Ajit Varma · Ram Prasad
Narendra Tuteja *Editors*

Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration

Fourth Edition

Springer

Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration

Ajit Varma • Ram Prasad • Narendra Tuteja
Editors

Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration

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 978-3-319-68866-4 ISBN 978-3-319-68867-1 (eBook)
https://doi.org/10.1007/978-3-319-68867-1

Library of Congress Control Number: 2017960850

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

Mycorrhizal symbioses, found in almost all ecosystems, are fundamental to improve plant fitness and soil quality through key ecological processes. Mycorrhizal fungi have been shown to fulfil a broad spectrum of beneficial functions for their host plants as well as for their environments like improved nutrient and water uptake, enhanced tolerance against biotic and abiotic stresses and improved soil structure to counteract soil erosion. In the last decade, it has been observed that intensive agricultural practices like the use of large amounts of mineral fertilizers and pesticides can have severe effects on arbuscular mycorrhizal fungi and other fungal symbionts in soils and, consequently, can negatively impact plant nutrition and growth. In fact, the loss of fungal diversity disrupts major ecosystem services such as ecosystem variability and productivity and might decrease plant biodiversity and, therefore, lead to land degradation.

The book "Mycorrhiza: Nutrient Uptake, Biocontrol, Ecorestoration" comes out in a period of time of exceptionally rapid growth in research on the role that mycorrhizal symbiosis has in plants to overcome biotic and abiotic stress and gives an excellent overview of the current knowledge in this field. It covers

different studies on plant nutrition by fungal strategies in nutrient-limiting environments and for sustainable agriculture. Several chapters describe mechanisms of mycorrhizal fungi to control different plant pathogens and increase plant health. Most chapters explain how mycorrhizal fungi inoculation has grown to be a biotechnological tool that is widely applicable in ecological restoration of degraded lands, in alleviating drought and heavy metal tolerances and in phytoremediation processes. The book is mainly focused on arbuscular mycorrhiza but also other types of interactions including those formed by orchid mycorrhizal fungi and endophytes and, to a lesser extent, by ectomycorrhizal fungi. I therefore congratulate the editors who have gathered so many different aspects of basic, biotechnological and applied research in the use of mycorrhizal symbiosis in human practices to preserve our environment, respecting nature and making a better world.

This third volume of the fourth edition of Mycorrhiza book culminates the work of compiling the most outstanding advances made on mycorrhizal symbiosis in the last decade. Many brilliant researchers have contributed with their chapters in the different editions to give a global and integrative vision of all this knowledge. There is no doubt that Prof. Ajit Varma has been the engine of bringing us together and integrating all this mycorrhizal knowledge during the last 25 years. I would like to take this opportunity to thank him for his huge effort and to the Springer Publishing House for relying on him and his undeniable tenacity and vision for the future.

It is hoped that this book is welcomed among senior scientists, academicians, young scientists and students, who should enjoy it for helping to understand, better and better, the functioning of this smart symbiosis and to stimulate further innovation and progress.

Murcia, Spain
12 October 2017

Asunción Morte

Preface

German pathologist A.B. Frank (1885) coined the term Mycorrhiza which literally means fungus roots. These fungi support the productivity of plants *via* the formation of dynamic associations with nutrient uptake via 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 assess numerous opportunities using modern tools of microbial biotechnology. The possibilities of genetically manipulating these associations have led to the optimization of plant productivity in ecosystems with minimal risk of environmental damage.

The fourth edition of the mycorrhiza book gives exemplary insight into the advancements in mycorrhizal studies. This edition extensively illuminates the nutrient uptake, biocontrol and eco-restoration of mycorrhizal association. The ability of mycorrhiza to provide resistance against various abiotic and biotic stresses has been explored in this edition. The mycorrhizal association is the state of the large majority of plants under most ecological conditions, and these symbioses are a strategic factor in ecosystem functioning. In established mycorrhizal associations, bidirectional exchange of nutrients and other benefits that occur require the formation of symbiotic interfaces resulting from morpho-physiological alterations in both plants and fungal tissues. Ecological disturbance, whether by natural or human activity, also influences the diversity of mycorrhizal fungi, and ecological resilience of these symbionts is essential for sustaining productivity. In conclusion, the efficient management of mycorrhizal systems has the potential to favour the sustainable production of quality foods while ensuring environmental quality for future generations.

It is hoped that this fourth 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 26 chapters covering the diverse mycorrhizal associations by 84 eminent academicians and subject specialists.

We are grateful to the many people who helped to bring this volume to light. We wish to thank Drs. 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 students Ms. Soumya Singh, Jaagriti Tyagi, and Monika and other technical staff.

Amity University, Uttar Pradesh, Noida, India

Ajit Varma
Ram Prasad
Narendra Tuteja

Contents

List of Contributors

Alok Adholeya The Energy and Resources Institute, New Delhi, India

Javaid Ahmad Government Model Science College, Jiwaji University, Gwalior, Madhya Pradesh, India

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

Plant Pathology Unit, Plant Protection Department, Desert Research Center, Cairo, Egypt

Manpreet Kaur Attri Amity Institute of Microbial Technology, Amity University, Noida, India

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

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

Purnima Bhandari Department of Botany, Panjab University, Chandigarh, India

Rouf Ahmad Bhat Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

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

Anil Bhushan Division of Vegetable Science and Floriculture, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Luís Carvalho Faculdade de Ciências, Centre for Ecology, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal

Patrícia Correia Faculdade de Ciências, Centre for Ecology, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal

Cristina Cruz Faculdade de Ciências, Centre for Ecology, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal

Surbhi Dabral Amity Institute of Microbial Technology, Amity University, Noida, India

Manab Das The Energy and Resources Institute, New Delhi, India

Moonisa Aslam Dervash Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Teresa Dias Faculdade de Ciências, Centre for Ecology, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal

Ahlan Sabah Ferdous Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

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

Bhoopander Giri Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi, India

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

RK Gupta Division of Vegetable Science and Floriculture, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Mohammad Riazul Islam Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

Vijay Sandeep Jakkula The Energy and Resources Institute, New Delhi, India

Iqbal Ahmad Bhat Javeed Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Dhruva Kumar Jha Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati, Assam, India

Puspa Raj Joshi Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

Lakita Kashyap Department of Botany, Panjab University, Chandigarh, India

Haseena Khan Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh

Mohammad Haneef Khan Joy Michelle Bergelson Lab, Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA

Murugan Kumar ICAR—National Bureau of Agriculturally Important Microorgansims, MauNath Bhanjan, Uttar Pradesh, India

Maya Kumari Directorate General of Life Sciences, Defence Research and Development Organization, New Delhi, India

Rafiq Lone Department of Botany, SBBS University, Khiala, Jalandhar, Punjab, India

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

Sarita Manandhar Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

Javid Manzoor Department of Environmental Science, Jiwaji University, Gwalior, India

M.K. Meghvansi Biotechnology Division, Defence Research & Development Establishment, Gwalior, Madhya Pradesh, India

Juliana Melo Faculdade de Ciências, Centre for Ecology, Evolution and Environmental Changes (cE3c), Universidade de Lisboa, Lisboa, Portugal

Shafa A. Mir Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Annu Mishra Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India

Siddhartha Kumar Mishra School of Biological Sciences, Dr. Harisingh Gour Central University, Sagar, India

Aneesul Mehmood Mohammad Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Rajalakshmi Muralidharan Department of Biotechnology, Ministry of Science & Technology, New Delhi, India

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

Ibrahim Ortaş Faculty of Agriculture, Department of Soil Science and Plant Nutrition, University of Çukurova, Adana, Turkey

Sushma Pandey Central Department of Botany, Tribhuvan Universtiy, Kathmandu, Nepal

Bijaya Pant Central Department of Botany, Tribhuvan Universtiy, Kathmandu, Nepal

Kamil Prajapati Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

Kamal Prasad Symbiosis Sciences Pvt Ltd., Gurgaon, India

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

Ramesh Namdeo Pudake Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India

Mazhar Rafique Faculty of Agriculture, Department of Soil Science and Plant Nutrition, University of Çukurova, Adana, Turkey

Faculty of Biological Sciences, Department of Plant Science, Quaid-I-Azam University, Islamabad, Pakistan

Vijay Raina Division of Vegetable Science and Floriculture, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Asmat Rashid Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Prasun Ray Noble Research Institute, Ardmore, OK, USA

Zafar A. Reshi Department of Botany, University of Kashmir, Srinagar, Jammu and Kashmir, India

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

Anil Kumar Saxena ICAR—National Bureau of Agriculturally Important Microorgansims, MauNath Bhanjan, Uttar Pradesh, India

Bhawna Saxena Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi, India

Sujit Shah Central Department of Botany, Tribhuvan Universtiy, Kathmandu, Nepal

Anil Kumar Sharma Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar, India

Bidisha Sharma Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati, Assam, India

Suvigya Sharma Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar, India

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

Roshni Shreastha Central Department of Botany, Tribhuvan University, Kathmandu, Nepal

Razia Shuab Department of Botany, University of Kashmir, Srinagar, Jammu and Kashmir, India

Sazada Siddiqui Biology Department, College of Science, King Khalid University, Abha, Saudi Arabia

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

Dig Vijay Singh Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Ishwar Singh Department of Botany, Hansraj College, University of Delhi, Delhi, India

Sandeep Singh Department of Botany, Panjab University, Chandigarh, India

Saumya Singh Amity Institute of Microbial Technology, Amity University, Noida, India

Bhat Mohd Skinder Department of Environmental Sciences, University of Kashmir, Hazratbal Srinagar, India

M. Nayeem Sofi Division of Soil Science, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir, India

Priyanka Srivastava Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Eram Sultan Laboratory of Plant Immunity, Department of Life Science, NIT, Rourkela, Odisha, India

Neha Nancy Toppo Central Tasar Research and Training Institute (Central Silk Board), Ministry of Textiles, GOI, Ranchi, Jharkhand, India

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

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

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

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

VS Verma Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu, Jammu and Kashmir, India

Shanquan Wang School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou, China

Khursheed Ahmad Wani Department of Environmental Science, ITM University, Gwalior, India

Chapter 1
The Mechanisms of Nutrient Uptake by Arbuscular Mycorrhizae

Ibrahim Ortaş and Mazhar Rafique

Abstract Mycorrhizal fungi are one of the commonly occurring living organism in soil facilitating plants in growth, development, stress tolerance, soil pollutants remediation, C-sequestration, food security and agricultural sustainability. Mycorrhizal fungi assist the plants in nutrient absorption by extending mycorrhizal hyphae network beyond the rhizosphere. Mycorrhizal inoculation alters the root architecture and studies showed that nutrient absorption capacity of inoculated root is much better than non-inoculated. For a long time, it is assumed that roots absorb nutrients only through direct pathway (DP) only while contribution of AM fungi in nutrients uptake by mycorrhizal pathway (MP) has been ignored. But now the development in scientific methods and tools, enabled the researcher to explore MP mechanism for macro and micro nutrients, moreover suppression of heavy metal stress to the plants. Besides that, mycorrhizal fungi obtain around 20% of photosynthesized C from the plant in exchange of nutrients. Moreover, this C triggers nutrient uptake and their translocation. Plant hormones and root exudates also influence the infection formation and development, they also point out new sites for the interaction of mycorrhizal fungi and plant roots. Nutrient mobility by MP is more secure and economical than DP. Understanding about the nutrient exploration, mobilization, and uptake in root-mycorrhizal interaction has been discussed here at molecular level. Contribution of plant and mycorrhizal transporters have been discussed which need further understanding. Also contribution of mycorrhizal inoculation on nutrient uptake compared with non-inoculated roots were discussed.

I. Ortaş (✉)
Department of Soil Science and Plant Nutrition, University of Çukurova, Faculty of Agriculture, Adana, Turkey
e-mail: iortas@cu.edu.tr

M. Rafique
Department of Soil Science and Plant Nutrition, University of Çukurova, Faculty of Agriculture, Adana, Turkey

Department of Plant Science, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_1

1.1 Introduction

Mycorrhizal fungi is one of the commonly occurring heterogeneous group of biological organism in soil interacting with broad range of plants (~80–90%) such as tropical forests, grasslands, alpine and crop lands (Bonfante and Genre 2010; Smith and Read 2008). They are colonized by mycorrhizal fungi and regulate nutrients uptake along with exchange of carbon. Arbuscular mycorrhizal (AM) fungi have evolutionized into living fossil in 460 million years by benefiting the plants confirmed by DNA sequence data recently (Redecker et al. 2000). In cropland, establishment of mycorrhizal association demonstrated the plant growth through nutrient uptake and favouring AM fungi in transporting photosynthesized C (Ortas et al. 2001). According to an estimate, 20% of the carbon fixed by plant is transferred to fungus (Parniske 2008). The AM fungal hyphae are extended beyond the accessibility limit of roots to transport immobile nutrients (P, Cu, Zn and partly NH_4) in soil which are not taken up by non-inoculated plants (Marschner 2012; Ortas 2003). Besides that, AM fungi work in poor soils to uptake and transport nutrients (macro and micro), although mycorrhizal association role related to carbon flux is less defined (Kayama and Yamanaka 2014; Selosse and Roy 2009). The AM fungi are obligate biotrophs and strictly dependent on green plants for carbohydrates availability because they lack the ability to absorb carbohydrates from other sources except the plant cells. The hyphal diameter ranges 2–20 μm with capacity of absorbing nutrients from area of 25 cm around the roots for translocation make the AM fungi variable and adaptable to various climatic conditions (Jansa et al. 2003; Munkvold et al. 2004).

Several studies have been conducted which sowed the functional diversity of AM fungi with host plant which impart significant impression on plant nutritional status, morphology, genetic expression and symbiotic efficiency in symbiotic relation (Jansa et al. 2008; Pellegrino and Bedini 2014; Prasad et al. 2017). The mycorrhizal functional diversity is an evolutionary process which faced various competitions inside and outside the host plant (Engelmoer et al. 2014). Specificity of the mycorrhizal fungi with plant species, always remained a key factor in garnering the benefits. It is assumed that monoculture inoculation is more advantageous than cocktail of the mycorrhizal fungi (Jansa et al. 2008). However, under greenhouse conditions indigenous, dual and mix cocktail mycorrhizae significantly inoculated mycorrhizae dependent citrus plant roots and increased nutrient uptake (Ortas et al. 2015; Ortas and Ustuner 2014).

Plants grown in artificial non-symbiotic conditions have shown that AM fungi significantly contribute to the uptake of soil nutrients, increase plant biomass and confer on the plant improved resistance to stress and pathogens (Smith and Read 2010). AM fungi do not only improve the transport of nutrients into the plant but can additionally, protect their host plants from toxicity by decreasing uptake of heavy metals on contaminated soils. This chapter includes the molecular mechanisms involved in uptake nutrients through root-mycorrhizal fungi symbiotic relation.

1.2 Mycorrhizal Symbiosis: A Mutualistic Niche

Niche is a central concept of ecology. In the early history of biology, mutually beneficial cooperation between species was recognized and later on several studies conducted on mycorrhizal symbiosis endorsed mutualism (De Bary 1879; Smith and Read 2010; West et al. 2007). With passage of time, technical advancements in the molecular and genetics knowledge facilitated thorough investigation of mutualistic relationship for enigmatic organisms including bacteria and fungi which ascertain variety of mutualistic interactions (Horton and Bruns 2001; Márquez et al. 2007). Despite the presence of antagonistic interaction among various organisms, mutualism in mycorrhizal association attained common and important recognition which is present in 80–90% of all extant of species and evolved nearly 500 Mya (Field et al. 2015; Redecker et al. 2001).

Relatively few studies have been quantified to evaluate the dimension of mutualism niche due to ambiguity of defining niche, although expansion potential of mutualistic niche is huge. To clarify the ambiguity of niche, a contemporary framework has been established by Chase and Leibold (2003) in contemporary niche theory (CNT). According to CNT, niche has two main components such as *requirement niche*: minimum environmental conditions necessary for organisms to maintain their population and *impact niche*: effect of organisms on respective minimum environmental conditions. This framework can be used as footstep for the years old concept of symbiotic niche between plants and AM fungi for resource allocation, consumption and modification of environments where they occur (Fig. 1.1).

Impact niche changes by mutualism. For instance, some plants alone can uptake the organic N-sources such as glycine and amino acids, whereas plants with mycorrhizal association absorb greater fraction of organic N. Similarly, resource consumption ratios may also alter by stoichiometric response of plant tissues to the mycorrhizal colonization (Güsewell 2004).

1.3 Symbiotically Developed Root Modifications for Nutrient Uptake

Plants adapt their phenotype according to the surrounding environmental changes due to stress (heat, nutrient, salinity, drought and osmotic stress), nutrient availability and mutualistic association with mycorrhizal fungi and bacteria (Nath et al. 2016). If we talk about the plant-roots phenotypic modification, they are mainly influenced by bacterial and mycorrhizal colonization and nutrients availability.

Nutrient availability is a fundamental signal in root architecture. Plant nutrients in soil such as phosphate and nitrate can be perceived as signals which trigger molecular mechanism of plant in modifying cell division, and differentiation process of roots. It alters the root system architecture in general. Internal and

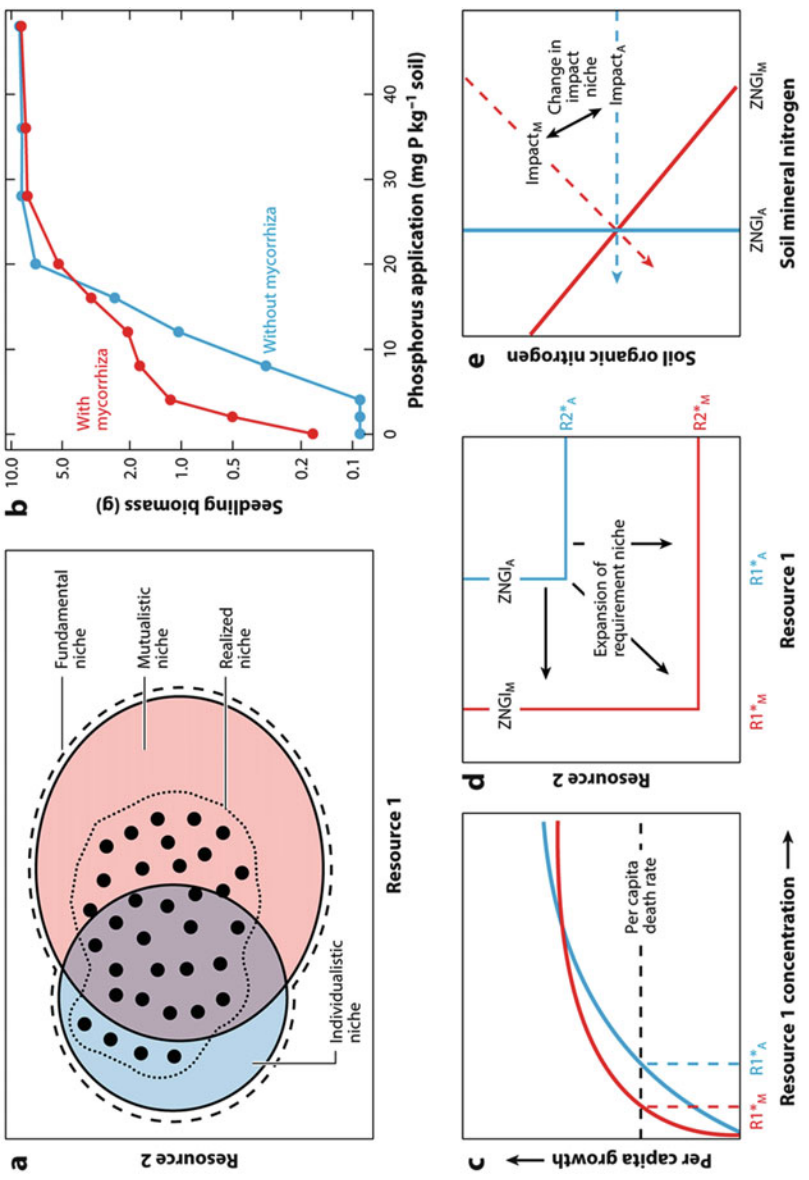


Fig. 1.1 Defining the mutualistic niche. (**a**) Hutchinson's fundamental niche (*dashed line*) and realized niche (*dots within dotted line*) distinguish potentially suitable and occupied niche space, respectively. (**a**) Possible contribution of mutualism (*red*) to what is generally considered fundamental niche space.

external concentration of nutrients affect the sensitivity of root formation and alters development processes of primary root growth, lateral root formation and root hair development. Besides that, growth regulators (cytokinin, ethylene and auxin) also subsidise in the root architecture as the hormone synthesis and transportation mediate root development (López-Bucio et al. 2003).

The AM colonized roots are apparently having recognizable lateral root branches, root hairs and root apex as the non-mycorrhizal roots (Berta et al. 1995). Mycorrhiza colonized plants have root hairs like non-mycorrhizal, although their density and the length are different (Orfanoudakis et al. 2010). Non-mycorrhizal plants get nutrients by direct pathway (DP) from soil-root interface in comparison to the mycorrhizal pathway (MP). In MP, nutrients are taken up and translocated rapidly through extraradical mycelium (ERM) to the intraradical mycelium (IRM). This process can take place up to many centimeters leading to the plant cell through interfacial appoplast. The orthophosphate (Pi) and NH_4^+ transporters are present in perifungal membrane which are expressed in AM fungi inoculated roots (Bucher 2007; Guether et al. 2009b; Smith and Smith 2011). Meanwhile, perifungal membrane surrounding the arbuscules and intracellular coils are energized by H^+-ATPase (Rosewarne et al. 2007). The DP absorbs nutrients from rhizosphere (immediate vicinity of roots) into the root epidermal and hair cells, whereas, mycorrhizal hyphae extended far away from the rhizosphere can transport nutrients to cortical cells considerably by MP (Fig. 1.2). It is highly regulated and rapid transit system if delivering nutrients.

Moreover, in stress conditions, AM fungal colonized roots alter their physiological and developmental growth to cope up the situation. For instance, in water stress condition, stress related genes in colonized roots express and form proteins to alleviate stress. Moreover, AM fungi also produce a large number of external mycelium to explore the soil for water uptake.

Fig. 1.1 (continued) (**b**) Growth rate for mycorrhizal (*red*) and nonmycorrhizal (*blue*) Eucalyptus seedlings across a phosphorous fertilization gradient. (**c**) A population model generalizing effect of phosphorus gradient, now termed resource 1 concentration, on per capita birth and death rates in the presence (*red*) and absence (*blue*) of mycorrhizae. This model can be used to determine the minimum resource requirements (R^*) of a species growing in mycorrhizal symbiosis (R^* M, *red*) versus those growing alone (R^* A, *blue*). Importantly, the model works for a plant growing with or without its mycorrhizal fungus or a fungus growing with or without its mycorrhizal host plant. (**d**) R^* can be quantified for multiple resources and used to predict how mycorrhizal symbiosis expands the requirement niche. *Lines* show how mutualism could expand the niche by shifting from the $ZNGI_{Alone}$ ($ZNGI_A$) to the $ZNGI_{Mutualism}$ ($ZNGI_M$), on the basis of the R^* for each of two essential non-substitutable resources, such as N and P. (**e**) Resource consumption (impact niche) may also change as a result of mutualism. *Dashed arrows* represent consumption vectors showing the trajectory along which resources will be drawn down for a species alone ($Impact_A$) or with a mutualist partner ($Impact_M$). In this example, mycorrhizal mutualism enables plant consumption of organic N (*red arrow*, $Impact_M$); in contrast, nonmycorrhizal plants can access only mineral N (*blue arrow*, $Impact_A$). The *diagonal red* $ZNGI_M$ line indicates that the two resources are substitutable for the mycorrhizal plant. Abbreviation: *ZNGI* zero net growth isocline (Peay 2016)

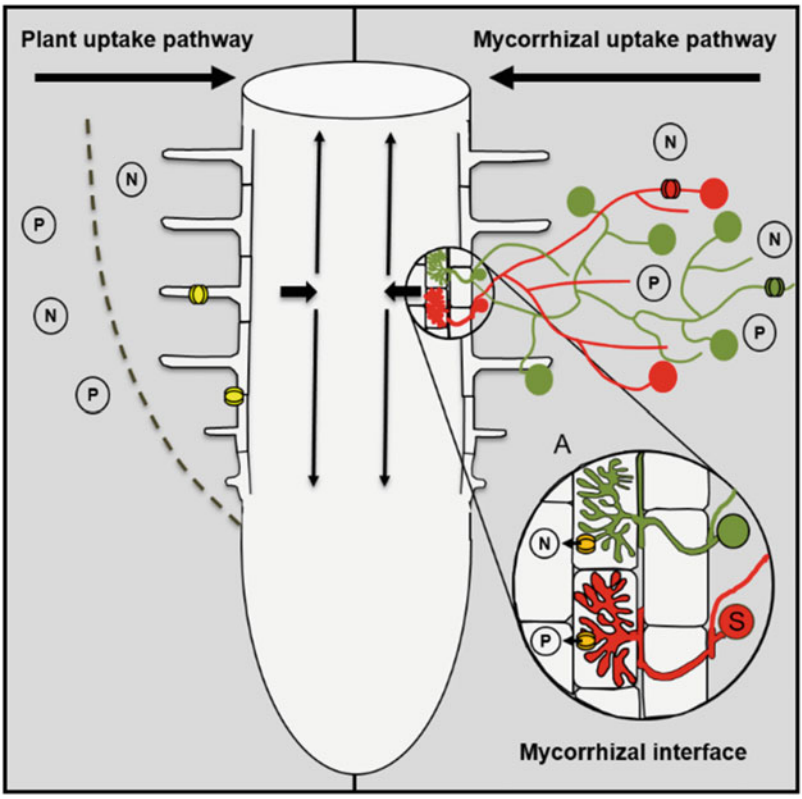


Fig. 1.2 Nutrients transportation by direct (plant) uptake pathway (DP) and mycorrhizal uptake pathway (MP). Plants can take up nutrients by transporters that are located in epidermis or root hairs (*yellow symbols*) or via the MP that comprises the uptake of nutrients by fungal transporters in the extraradical mycelium (*red* or *green symbols*), the transport through the hyphae from the ERM to the IRM (see mycorrhizal interface), and the uptake from the mycorrhizal interface by mycorrhiza-inducible plant transporters in the peri-arbuscular membrane (*orange symbols*). Indicated by the *red* and *green* fungal structures is the colonization of one host root by multiple fungal species that can differ in their efficiency with which they are able to take up nutrients from the soil and transfer these nutrients to their host (Bücking and Kafle 2015)

1.4 Hormonal Regulation and Mycorrhizal Symbiosis

Knowledge about the contribution of plant hormones in mycorrhizal symbiosis is a major breakthrough recently where they are believed to regulate colonization. Phytohormones perform differential functions to develop symbiotic relationship, some mediate pre-symbiotic signalling while other do morphological adaptations in root for fungus accommodation, proliferation and functionality. To evaluate the role of hormones, a study was conducted on pea plant by considering gibberellins and brassinosteroids. In gibberellin-deficient na-1 mutant, AM fungal colonization

was comparatively increased against wild-type plants. Besides that, application of GA3 reversed this process and mutant la cry-s lacking gibberellin signalling DELLA proteins reduced colonization. Such type of the changes is parallel with gene expression associated with mycorrhizal colonization. Moreover, mutant of brassinosteroid-deficient lkb did not alter colonization percentage. DELLA proteins produced in response of gibberellins production suppress arbuscule formation in roots of pea which shows the connection of plant hormones and mycorrhizal colonization in root cells (Foo et al. 2013). In another study, it was concluded that crosstalk between ABA-gibberellins and ABA-ethylene regulate arbuscule formation and AM fungi development, respectively (Gutjahr 2014).

Mycorrhizal spores have the capacity to germinate even in absence of host plant but the growth of hyphae remains restricted with short length. Further growth of the hyphae is dependent on signalling molecules released from the host plant which govern recognition in first stage between host and AM fungi. Before appressorium formation, AM fungi develop extensive branching in vicinity of host roots (Giovannetti et al. 1994). In a study conducted, strigolactone was isolated from *Lotus japonicus* root exudates, which is assumed to be responsible for hyphal branching. In AM fungus *Gigaspora margarita*, very low concentration of strigolactone can do extensive branching of hyphae in germinating spores (Akiyama et al. 2005). Moreover, influence of shoot and root borne cytokinin on bidirectional exchange of P and C is explained (Fig. 1.3).

1.5 Mechanism of Nitrogen Uptake by Mycorrhizal Pathway

For a long time, N transfer from soil to plant by MP remained unimportant on the premises that plant roots can uptake inorganic N as NO_3^- and NH_4^+ conveniently due to their mobility in soil and organic N may not be available to the AM fungi. Considerable amount of NO_3^- and NH_4^+ (approximately 20–50 μM) is present in unfertilized soils but due to high mobility, they are not depleted in rhizosphere (McDowell et al. 2004). In rhizosphere, N forms NO_3^- and NH_4^+ selectively taken up by specific plant species and also possibly some of those plant species are mycorrhizae dependent. In generally since NH_4^+ is absorbed on soil and organic colloides the concentration in soil is low and less mobile. Possibly mycorrhizae phyhae absorbe NH_4^+ rather than NO_3^-. In scavenging and uptake of N by hyphae or plant roots are expected similar (Marschner and Rimmington 1988). The studies conducted on leguminous plants showed that, AM fungi inoculated plants increased total N uptake in comparison to non-inoculated plants (Azcon et al. 1992; Smith and Read 2008). Some recent developments have been observed in non-leguminous plants also where AM fungi assisted in N uptake (Johnson 2010). Number of studies have been conducted by providing access to ERM in compartmented pots with labelled N ($15NO_3^-$ or $15NH_4^+$). Results showed that AM fungi inoculated plants

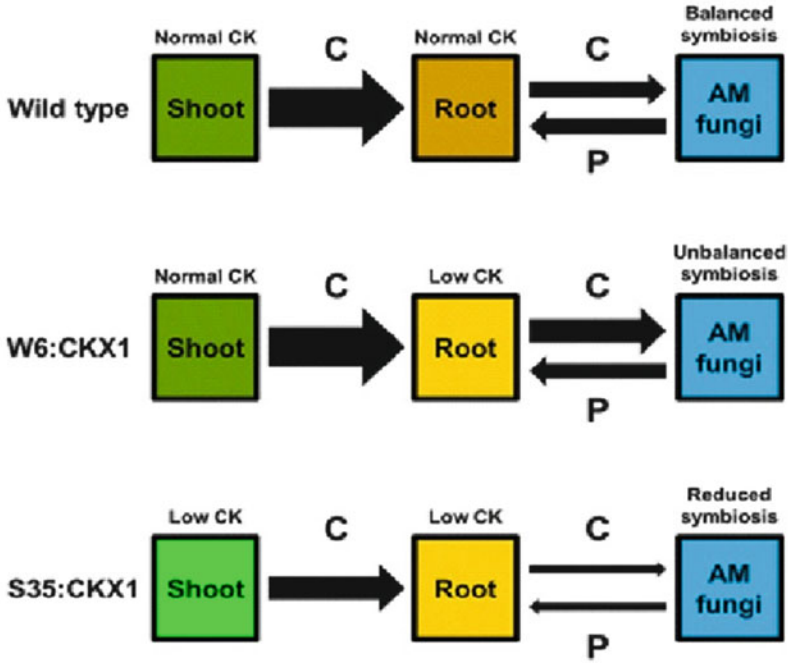


Fig. 1.3 Proposed model for the cytokinin (CK) regulation of bidirectional exchange of C and P in AM symbiosis. A normal CK status in shoots and in roots contributes to balance bidirectional flow of C and P between symbionts. A normal CK status of the shoots combined with reduced CK status in the roots maintain a strong source of C from the shoots into the roots but may reduce sink capacity of roots in relation to that of AM fungi, irrespective of P supply, causing an unbalanced C for P exchange between symbionts. A strongly reduced CK status of shoots negatively regulates the source of C from shoots by reducing the availability of sugars, which may reduce the AM pathway for P uptake, irrespective of the root CK status. *Arrow thickness* illustrates the relative flow strength of C or P (Cosme et al. 2016)

transferred more 15N than non-inoculated (Azcon et al. 1992; Tanaka and Yano 2005). Transportation of N is also dependent on moisture contents, NH_4^+ mobility is found greater than NO_3^- in suitable moisture conditions (Tanaka and Yano 2005). In Cucumber, AM fungi transferred 10% of total (Johansen et al. 1992) while on other side in tomato, this amount reached up to 42% (Mäder et al. 2000). In monoxenic culture of AM fungi, ERM take up N and make unit if amino acid in the form of arginine (*Arg*) and it is main form of N transported from ERM to IRM (Govindarajulu et al. 2005). Some studies showed that, N releases form the *Arg* as NH_4^+ before transferring to the root cells interface (Cruz et al. 2007). The pathway adapted (Jin et al. 2005), shows N movement from soil to the plant root through MP (Fig. 1.4).

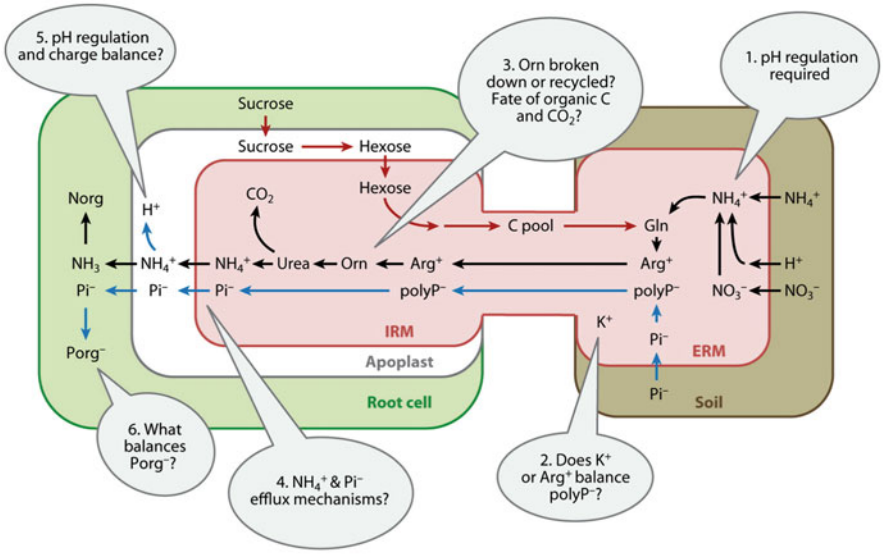


Fig. 1.4 Uptake of inorganic N into the ERM by synthesizing *Arg* which is positively charged (Arg^+), and its movement to the IRM and breakdown require concomitant charge balance. Complete breakdown of one Arg^+ would produce three NH_3 and one NH_4^+, with the charge of the latter balanced by whatever anion(s) balanced the original Arg^+ throughout its synthesis and delivery (Jin et al. 2005). Theoretically it would allow transfer of four N per P (molar basis), thus allowing contribution of approximately 18% of the total plant N, assuming a plant N: P mass ratio of approximately 10:1

1.6 Mechanism of Phosphorus Uptake

Recent research on nutrient uptake models shown that mycorrhizal fungi have strong potential to be the drivers of nutrient mobilization processes in some ecosystems. Mycorrhizal fungi are also involved in the mobilization of nitrogen and phosphorus from natural substrates (Read and Perez-Moreno 2003). Pi concentration in soil solution is usually less than 10 μM due to strong adsorption with Ca, Fe and Al at high and low pH (Schachtman et al. 1998). This low solubility in soil solution, also reduces mobility to root epidermis. The P nearby to roots is taken up and creates depletion zone where alternative P is not available du e to string bonding (Fig. 1.5). Different plant taxa may have different phosphorus demands. Plant taxa also have different root systems and some are strongly depend on mycorrhizae in term of P nutrition (Ortas 2012). If plants are mycorrhizae dependent for P nutrition, they are definitely required mycorrhizae infection. Mycorrhizae requirement depends on soil fertility and plant nutrient demand. Under greenhouse conditions P and Zn deficient soil was treated with several P and Zn levels with and without mycorrhizal inoculation. It has been found that at low level of P fertilization mycorrhizae inoculation significantly increased plant growth (Fig. 1.6) and P

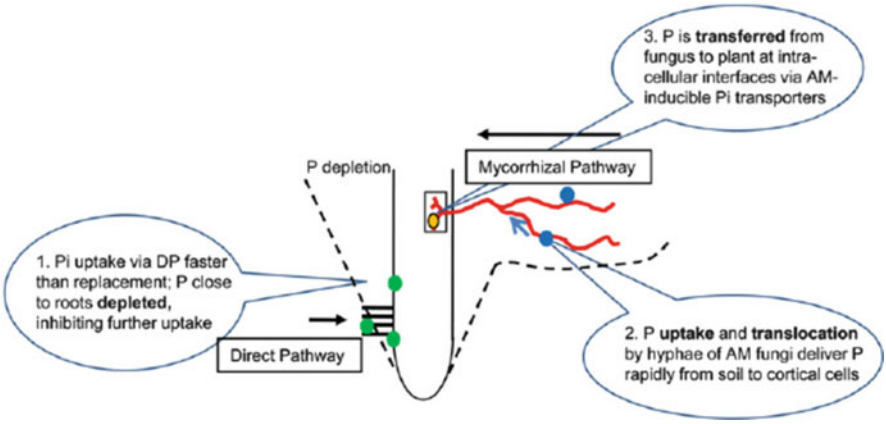


Fig. 1.5 The two pathways of P uptake in an AM root involve different regions of the root, different cell types, and different Pi transporters. In the DP, Pi is absorbed from the rhizosphere by plant Pi transporters in epidermis and root hairs (*green circles*) close to the root surface. Uptake is normally faster than replacement by diffusion from the bulk soil, resulting in reduced Pi concentrations close to the roots. In the MP, Pi is taken up into AM fungal hyphae by fungal Pi transporters (*blue circles*) several centimetres from the root and translocated to intracellular fungal structures in root cortical cells. Plant Pi transporters, induced in colonized cells (*yellow circle*), transfer Pi from the interfacial apoplast to plant cortical cells (Smith et al. 2011)

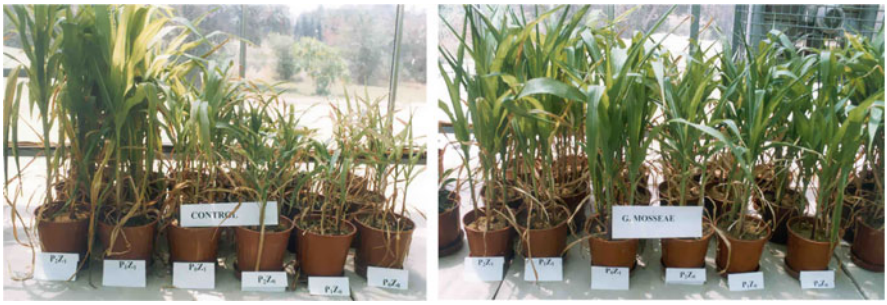


Fig. 1.6 Effect of P and Zn application on maize growth with and without mycorrhizal inoculation

uptake (Ortas 2012). And with high P fertilizer there were no differences in between mycorrhizal inoculation in term of plant growth and nutrient uptake. The improved uptake of P and Zn by mycorrhizal inoculation may be happened due to the extensive network of external hyphae accessing nutrients beyond the rhizosphere zone.

Some plant have root epidermis and root hairs could not follow DP to uptake P. Number of mechanisms including P solubilisation, exploration of soil beyond rhizosphere limit (Hinsinger 2001) and absorption of P into the mycorrhizal hyphae have been employed by inoculated plants. As a result, distant P becomes accessible to the inoculated plants which must diffuse to plant roots. If threshold concentration

required for P absorption is decreased, mycorrhizal affinity to P increase then movement of P can be enhanced in mycorrhizal hyphae (Koide 1991). Since nitrogen forms have strong influence on rhizosphere pH, and as a result rhizosphere pH have significant effects on nutrient uptake of mycorrhizal and non-mycorrhizal inoculated plants (Ortas et al. 1996). Phosphorus solubilizing bacteria and other microbes produce organic acids to solubilize the bounded P, which is taken up by mycorrhizal hyphae to transport plant roots (Bolan 1991). Soil surrounding the mycorrhizal hyphae show slower depletion zone formation (Koide 1991; Li et al. 1991; Silberbush and Barber 1983).

The Pi uptake through MP imitates through delivering to the ERM against electrochemical potential gradient, energized by H^+-ATPase's (Bucher 2007). As the Pi taken up, polyphosphate (polyP) buffers cytoplasmic Pi concentration accumulated in the hyphae where it is stored and translocated (Hijikata et al. 2010). PolyP has variable range of chain length, having soluble to insoluble behaviour. Its amount is strongly variable depending up on amount of P available and it develops translocation sites for the uptake in ERM (Viereck et al. 2004). As both Pi and Poly P are negatively charged, they are electronically balanced by K^+ and Mg^{+2} taken up from soil, but plants inoculated with mono-culture of AM fungi produce Arg^+ with polyP (Jin et al. 2005; Ryan et al. 2007). Plant Pi and AM fungal Pi transporters are similar to each other which could involve in efflux of Pi (Preuss et al. 2010).

Initial studies showed that mycorrhizal colonization does not have effect on DP of P uptake, and MP uptake contributes as an additive offer. Based on this assumption, P inflow was calculated differently in both uptake (Tinker 1984). MP was found very responsive for strongly mycorrhizal dependent plants such as leek, onion and clover. In one of the studies conducted on tomato plant, DP was found completely inactive while MP delivered 100% of the required P (Smith et al. 2004). These studies show the functional diversity of mycorrhizal symbioses in P uptake and delivery *via* both pathways (Facelli et al. 2010). When P uptake in tomato was observed *via* MP, against two different AM fungi inoculation, *Rhizophagus irregularis* was found more promising than *Gigaspora margarita*. Similarly, Ortas et al. (2013) reported that mycorrhizae species inoculated tomato plant P uptake was changed in between mycorrhizae species. Here considerable findings were noted that total concentration of P was same while DP and MP were found reciprocal (Facelli et al. 2010).

1.7 Mechanism of Potassium, and Sodium Uptake

Fungi has two groups of transporters named as HAK (high affinity K uptake) and Trk (transporter of K). These transporters are involved in transportation of K^+ and Na^+ (Benito et al. 2011; Corratgé-Faillie et al. 2010). Trk is found more frequent in genetic database of ECM fungi while this family is missing in AM fungus *R. irregularis* while HAK transporter was found for K-uptake (Garcia and Zimmermann 2014). It is assumed that K exporters in AM fungi are specifically

expressed, localized and regulated at interface. Two types of ion channels are supposed to be responsible for this role i.e. TOK (tandem-pore outward K channel) and SKC (shaker-like K channel) (Garcia and Zimmermann 2014). In *R. irregularis*, two putative SKC genes have been identified. Although TOK channels are not observed in *R. irregularis*, but they can release K during ECM. In plants, molecular data about K movement is still lacking but a putative K uptake (KUP) transporter has been observed showing 44-fold upregulation in mycorrhizal infected *Lotus japonicus* plant (Guether et al. 2009a). Lack of data in molecular mechanism needs further studies about K-uptake and fate. Under field conditions it has been observed mycorrhiza inoculated sweet corn plant have higher K concentration than that of non-inoculated one (Ortaş and Sari 2003).

1.8 Mechanism of Addressing Micronutrients and Heavy Metal

Heavy metal ions (Cu^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}) are essential micronutrients for plant growth and its metabolism, but excess of these can become extremely toxic. For instance, plants tightly control Fe homeostasis as deficiency and overload of Fe^{2+} is immediately shown in plants due to its high reactivity by Fenton reaction (Morrissey and Guerinot 2009). Root exudates have diverse chemical composition from inorganic (phosphates, protons, etc.) to organic (amino acids, carbohydrates, carboxylate anions, enzymes, phenolic, etc.) which alter the chemical composition of rhizosphere and micronutrients concentration. As the AM fungi is being evaluated for various stress alleviation, remediation of heavy metal polluted soils by AM fungi is also a newly establishing approach. Use of mycorrhizal fungi in polluted soils assist plants in growth promotion and heavy metals translocation to the shoots (Kamal et al. 2010). Several studies conducted recently which showed that various AM fungi, for instance *G. mosseae*, *R. irregularis* enhanced heavy metal translocation in the shoot with high efficiency (Ali et al. 2015; Zaefarian et al. 2013). Besides that, AM fungi also reported to regulate heavy metals translocation by considering plant health and assist the plants in avoiding metal toxicity (Cd, Mn and Zn) within the plants (Li and Christie 2001, Kamal et al. 2010), although some increase was also observed (Liao et al. 2003). Micronutrients such as Cu and Zn are diffusion-limited in soils where they are taken up by plants in assistance with mycorrhizal hyphae. In a study conducted on coffee plant under heavy Cu and Zn toxicity, it was concluded that AM fungi protects plant seedling from toxicity (Andrade et al. 2010).

Heavy metal toxicity can be illustrated in three different molecular mechanisms on basis of their physical and chemical properties such as: (a) relative oxygen species (ROS) production by Fenton reaction or autoxidation in transition metals (Cu^{2+} and Fe^{2+}), (b) essential functional groups blockage in biomolecules by non-redox-reactive heavy metals (Cd^{2+} and Hg^{2+}) and (c) essential metal ions

displacement form biomolecules. Plants exposure to Cu^{2+} and Fe^{2+} develops oxidative stress by H_2O_2 accumulation, lipid peroxidation and oxidative burst. Heavy metals, particularly Cd^{2+} causes oxidative enzymes inhibition, specifically glutathione reductase. Studies showed that toxicity of Cd^{2+} may trigger disturbance in redox control of plant cell, which leads to the series of action culminating to the plant growth hindrance. Moreover, stimulating secondary metabolism, lignification and ultimately plant cell death. Studies showed that, plants associated with mycorrhizal fungi were found less-sensitive to Cd^{2+} than non-mycorrhizal plants. Possibly, interaction of AM fungi and plants stimulate phenolic defence which is observed in *Paxillus-pinus*. Most of the Cd-induced changes in response of phenolic in root part of the symbiosis are buffered. AM fungi infected roots resist structural changes which could happen due to Cd^{2+} toxicity. There can be various possibilities to cope up mycorrhizal protected root injury due to Cd^{2+} by metal chelation, by defence system or limiting Cd^{2+} access to sensitive intra- or extracellular sites (Schützendübel and Polle 2002).

1.9 Carbon as Trigger for Nutrient Uptake and Transport in the AM Symbiosis

Carbon is a main element in symbiotic relation between plant and AM fungi, although transfer of C to AM fungi was established in 1960s, but its detailed molecular mechanism remained an important question for a long time (Smith and Read 2010). About 20% of the photosynthesized C is transferred to the AM fungi (Douds et al. 2000). The results of Kucey and Paul (1982) showed that AM colonization and Rhizobium inoculated plant roots resulted in doubling of the C allocation into the nodules and C allocation to roots in the plants colonized by and AM was greater (12% of recently fixed C) than for plants colonized only with mycorrhiza (4%).

Host plants transfer the C to AM fungi in the form of sucrose (hexose sugar) after process by acid sucrose synthase or invertase and taken up by high affinity monosaccharide transporter (Helber et al. 2011; Hohnjec et al. 2003; Schaarschmidt et al. 2006). AM fungi induce plant acid invertase expression as sucrose cannot be used as C source (Schaarschmidt et al. 2006). Exchange of C and P between host and AM fungi is directly proportional and facilitate each other. Besides that, C also acts as triggering element for uptake of fungal N and its transportation which is controlled by specific gene expression in the fungi (Fellbaum et al. 2012a). To exchange C and P, assistance of fungal monosaccharide transporter (*MST2*) and mycorrhiza-inducible plant P transporter (*Pt4*) are colocalized in AM fungi whose expression is strongly correlated (Helber et al. 2011). Expression of *Pt4* and transfer of phosphate are preconditions to establish successful symbiotic relationship and arbuscule formation (Javot et al. 2011). Arbuscules also degrade and re-form in the host, where N-deprivation can rescue degradation in *Pt4* mutants. Its shows that N, P and

C can mutually benefit when regulating intracellular colonization (Fellbaum et al. 2012b). Carbon transferred from plants is consumed in development of AM fungi biomass, extension of ERM, development of new arbuscules and in energy consumption processes. The IRM directly uptake glucose from the connection points, P uptake and transfer in AM fungi directly influenced by C supply (Kiers et al. 2011; Woolhouse 1975). In a study, it was concluded that external application of glucose to *Gigaspora margarita* stimulates Pi efflux from IRM (Solaiman and Saito 2001). Moreover, N uptake is also stimulated and transported in AM fungi due to C supply which are mediated by triggering fungal gene expression (Dietz et al. 2011). It influences the N-assimilation by biosynthesising *Arg* in ERM and exported to IRM where it induces urease and arginase activity boosting NH^+ level in IRM to facilitate N release into mycorrhizae (Bücking et al. 2012). Similarly, in another study C availability for *R. irregularis* on N-uptake and its transport in symbiotic relation was evaluated. The labelled ^{15}N and ^{14}C-arginine were used to track their movement AM fungi, their genetic expression and enzymatic activities were also monitored. Increase in C transport influenced N and *Arg* uptake with triggering genetic changes (Fellbaum et al. 2012a).

1.10 Conclusion

When considering mycorrhizal symbioses in global context of food security, sustainable agriculture, ecosystem conservation, developing plants for future needs and environmental change, mycorrhizal fungi is acknowledged as crucial factor in fields of C-sequestration, P and N uptake and mobilization with cycling of other nutrients required for plant survival. Capacity of AM fungi to extract nutrient beyond the rhizosphere is amazing and they release nutrients inside the plant with many expressions of plant transporters activating symbiotic relation. Amount of nutrient allocation between AM fungi and plants is still needed to explore and the environment which facilitates this exchange and allocation process should also be addressed. The plant transporters influenced by AM fungi have been well characterized but their new functions are being unveiled which should be explore i.e. nutrient sensing and their evolutionary process. It also influences root architecture, ling plant development with environmental conditions. Selection and optimization of mycorrhizal fungi according to the soil conditions is prerequisite for plant growth and sustainable agriculture.

References

Akiyama K, Matsuzaki K-I, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Ali N, Masood S, Mukhtar T, Kamran MA, Rafique M, Munis MFH, Chaudhary HJ (2015) Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal

uptake of *Linum usitatissimum* in association with *Glomus intraradices*. Environ Monit Assess 187(6):311. https://doi.org/10.1007/s10661-015-4557-8

Andrade SAL, Silveira APD, Mazzafera P (2010) Arbuscular mycorrhiza alters metal uptake and the physiological response of *Coffea arabica* seedlings to increasing Zn and Cu concentrations in soil. Sci Total Environ 408:5381–5391. https://doi.org/10.1016/j.scitotenv.2010.07.064

Azcon R, Gomez M, Tobar R (1992) Effects of nitrogen-source on growth, nutrition, photosynthetic rate and nitrogen-metabolism of mycorrhizal and phosphorus-fertilized plants of *Lactuca sativa* L. New Phytol 121:227–234

Benito B, Garciadeblás B, Fraile-Escanciano A, Rodríguez-Navarro A (2011) Potassium and sodium uptake systems in fungi. The transporter diversity of *Magnaporthe oryzae*. Fungal Genet Biol 48:812–822

Berta G, Trotta A, Fusconi A, Hooker JE, Munro M, Atkinson D, Giovannetti M, Morini S, Fortuna P, Tisserant B, Gianinazzipearson V, Gianinazzi S (1995) Arbuscular mycorrhizal induced changes to plant-growth and root-system morphology in *Prunus cerasifera*. Tree Physiol 15:557–557

Bolan N (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol 173:11–26

Bücking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy 5:587–612

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. Intech, Rijeka

Chase JM, Leibold MA (2003) Ecological niches: linking classical and contemporary approaches. University of Chicago Press, Chicago, IL

Corratgé-Faillie C, Jabnoune M, Zimmermann S, Véry A-A, Fizames C, Sentenac H (2010) Potassium and sodium transport in non-animal cells: the Trk/Ktr/HKT transporter family. Cell Mol Life Sci 67:2511–2532

Cosme M, Ramireddy E, Franken P, Schmülling T, Wurst S (2016) Shoot- and root-borne cytokinin influences arbuscular mycorrhizal symbiosis. Mycorrhiza 26:709–720

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loução MA, Jakobsen I (2007) Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhizal fungi. Plant Physiol 144:782–792

De Bary A (1879) Die erscheinung der symbiose. Verlag von Karl J, Trübner

Dietz S, von Bülow J, Beitz E, Nehls U (2011) The aquaporin gene family of the ectomycorrhizal fungus Laccaria bicolor: lessons for symbiotic functions. New Phytol 190:927–940

Douds DDJ, Pfeffer PE, Shachar HY (2000) Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: Kapulnik Y, Douds DDJ (eds) Arbuscular mycorrhizas: physiology and function. Kluwer Academic, Dordrecht

Engelmoer DJ, Behm JE, Toby Kiers E (2014) Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance. Mol Ecol 23:1584–1593

Facelli E, Smith SE, Facelli JM, Christophersen HM, Andrew Smith F (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytol 185:1050–1061

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bücking H (2012a) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 109:2666–2671

Fellbaum CR, Mensah JA, Pfeffer PE, Kiers ET, Bücking H (2012b) The role of carbon in fungal nutrient uptake and transport: implications for resource exchange in the arbuscular mycorrhizal symbiosis. Plant Signal Behav 7:1509–1512

Field KJ, Rimington WR, Bidartondo MI, Allinson KE, Beerling DJ, Cameron DD, Duckett JG, Leake JR, Pressel S (2015) First evidence of mutualism between ancient plant lineages (*Haplomitriopsida liverworts*) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO_2. New Phytol 205:743–756

Foo E, Ross JJ, Jones WT, Reid JB (2013) Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. Ann Bot 111:769–779

Garcia K, Zimmermann SD (2014) The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci 5:337. https://doi.org/10.3389/fpls.2014.00337

Giovannetti M, Sbrana C, Logi C (1994) Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytol 127:703–709

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Guether M, Balestrini R, Hannah M, He J, Udvardi MK, 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, Neuhäuser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P (2009b) A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. Plant Physiol 150:73–83

Güsewell S (2004) N:P ratios in terrestrial plants: variation and functional significance. New Phytol 164:243–266

Gutjahr C (2014) Phytohormone signaling in arbuscular mycorhiza development. Curr Opin Plant Biol 20:26–34

Helber N, Wippel 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

Hijikata N, Murase M, Tani C, Ohtomo R, Osaki M, Ezawa T (2010) Polyphosphate has a central role in the rapid and massive accumulation of phosphorus in extraradical mycelium of an arbuscular mycorrhizal fungus. New Phytol 186:285–289

Hinsinger P (2001) Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant Soil 237:173–195

Hohnjec N, Perlick AM, Pühler A, Küster H (2003) The Medicago truncatula sucrose synthase gene MtSucS1 is activated both in the infected region of root nodules and in the cortex of roots colonized by arbuscular mycorrhizal fungi. Mol Plant Microbe Interact 16:903–915

Horton TR, Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Mol Ecol 10:1855–1871

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

Javot H, Penmetsa RV, Breuillin F, Bhattarai KK, Noar RD, Gomez SK, Zhang Q, Cook DR, Harrison MJ (2011) Medicago truncatula mtpt4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. Plant J 68:954–965

Jin H, Pfeffer P, Douds D, Piotrowski E, Lammers P, Shachar-Hill Y (2005) The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. New Phytol 168:687–696

Johansen A, Jakobsen I, Jensen E (1992) Hyphal transport of 15N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol 122:281–288

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol 185:631–647

Kamal S, Prasad R, Varma A (2010) Soil microbial diversity in relation to heavy metals. In: Sherameti I, Varma A (eds) Soil heavy metals. Springer, Berlin, pp 31–64

Kayama M, Yamanaka T (2014) Growth characteristics of ectomycorrhizal seedlings of *Quercus glauca*, *Quercus salicina*, and *Castanopsis cuspidata* planted on acidic soil. Trees Struct Funct 28:569–583. https://doi.org/10.1007/s00468-013-0973-y

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882

Koide RT (1991) Nutrient supply, nutrient demand and plant-response to mycorrhizal infection. New Phytol 117:365–386

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

Li XL, Christie P (2001) Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42:201–207

Li XL, George E, Marschner H (1991) Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil 136:41–48

Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50:847–853. https://doi.org/10.1016/s0045-6535(02)00229-1

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287

Mäder P, Vierheilig H, Streitwolf-Engel R, Boller T, Frey B, Christie P, Wiemken A (2000) Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. New Phytol 146:155–161

Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ (2007) A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science 315:513–515

Marschner P (2012) Marschner's mineral nutrition of higher plants. Academic, London

Marschner H, Rimmington G (1988) Mineral nutrition of higher plants. Plant Cell Environ 11:147–148

McDowell WH, Magill AH, Aitkenhead-Peterson JA, Aber JD, Merriam JL, Kaushal SS (2004) Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution. Forest Ecol Manag 196:29–41

Morrissey J, Guerinot ML (2009) Iron uptake and transport in plants: the good, the bad, and the ionome. Chem Rev 109:4553–4567

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. New Phytol 164:357–364

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. https://doi.org/10.3389/fpls.2016.01574

Orfanoudakis M, Wheeler CT, Hooker JE (2010) Both the arbuscular mycorrhizal fungus Gigaspora rosea and Frankia increase root system branching and reduce root hair frequency in *Alnus glutinosa*. Mycorrhiza 20:117–126

Ortas I (2003) Effect of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Harran Plain in South Anatolia. J Plant Nutr 26:1–17. https://doi.org/10.1081/pln-120016494

Ortas I (2012) Do maize and pepper plants depend on mycorrhizae in terms of phosphorus and zinc uptake? J Plant Nutr 35:1639–1656. https://doi.org/10.1080/01904167.2012.698346

Ortaş I, Sari N (2003) Enhanced yield and nutrient content of sweet corn with mycorrhizal inoculation under field conditions. Agricoltura Mediterranea 3–4:188–195

Ortas I, Ustuner O (2014) The effects of single species, dual species and indigenous mycorrhiza inoculation on citrus growth and nutrient uptake. Eur J Soil Biol 63:64–69. https://doi.org/10.1016/j.ejsobi.2014.05.007

Ortas I, Harris PJ, Rowell DL (1996) Enhanced uptake of phosphorus by mycorrhizal sorghum plants as influenced by forms of nitrogen. Plant Soil 184:255–264. https://doi.org/10.1007/bf00010454

Ortas I, Kaya Z, Çakmak I (2001) Influence of VA-mycorrhiza inoculation on growth of maize and green pepper plants in phosphorus and zinc deficient soils. Kluwer Academic, Dordrecht

Ortas I, Sari N, Akpinar C, Yetisir H (2013) Selection of arbuscular mycorrhizal fungi species for tomato seedling growth, mycorrhizal dependency and nutrient uptake. Eur J Hortic Sci 78:209–218

Ortas I, Akpinar C, Demirbas A (2015) Effect of mycorrhizal species on growth and nutrient uptake by seedlings of Citrus (*Citrus sinensis*) under three soil growth conditions. Curr Hortic 3:61–64

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Peay KG (2016) The mutualistic niche: mycorrhizal symbiosis and community dynamics. Annu Rev Ecol Evol Systemat 47:143–164

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

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

Preuss CP, Huang CY, Gilliham M, Tyerman SD (2010) Channel-like characteristics of the low-affinity barley phosphate transporter PHT1; 6 when expressed in Xenopus oocytes. Plant Physiol 152:1431–1441

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? New Phytol 157:475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921. https://doi.org/10.1126/science.289.5486.1920

Redecker D, Szaro TM, Bowman RJ, Bruns TD (2001) Small genets of Lactarius xanthogalactus, Russula cremoricolor and Amanita francheti in late-stage ectomycorrhizal successions. Mol Ecol 10:1025–1034

Rosewarne GM, Smith FA, Schachtman DP, Smith SE (2007) Localization of proton-ATPase genes expressed in arbuscular mycorrhizal tomato plants. Mycorrhiza 17:249–258

Ryan MH, McCully ME, Huang CX (2007) Relative amounts of soluble and insoluble forms of phosphorus and other elements in intraradical hyphae and arbuscules of arbuscular mycorrhizas. Funct Plant Biol 34:457–464

Schaarschmidt S, Roitsch T, Hause B (2006) Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots. J Exp Bot 57:4015–4023

Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus uptake by plants: from soil to cell. Plant Physiol 116:447–453

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Selosse M-A, Roy M (2009) Green plants that feed on fungi: facts and questions about mixotrophy. Trends Plant Sci 14:64–70. https://doi.org/10.1016/j.tplants.2008.11.004

Silberbush M, Barber S (1983) Sensitivity of simulated phosphorus uptake to parameters used by a mechanistic-mathematical model. Plant Soil 74:93–100

Smith S, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic, Elsevier, Cambridge

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic, San Diego, CA

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, 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 or total P uptake. New Phytol 162:511–524

Smith SE, Jakobsen I, Grønlund 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

Solaiman M, Saito M (2001) Phosphate efflux from intraradical hyphae of *Gigaspora margarita* in vitro and its implication for phosphorus translocation. New Phytol 151:525–533

Tanaka Y, Yano K (2005) Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant Cell Environ 28:1247–1254

Tinker PB (1984) The role of microorganisms in mediating and facilitating the uptake of plant nutrients from soil. Plant Soil 76:77–91. https://doi.org/10.1007/bf02205569

Viereck N, Hansen PE, Jakobsen I (2004) Phosphate pool dynamics in the arbuscular mycorrhizal fungus *Glomus intraradices* studied by in vivo31P NMR spectroscopy. New Phytol 162:783–794

West SA, Griffin AS, Gardner A (2007) Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. J Evol Biol 20:415–432

Woolhouse H (1975) Membrane structure and transport problems considered in relation to phosphorus and carbohydrate movements and the regulation of endotrophic mycorrhizal associations. In: Endomycorrhizas; Proceedings of a symposium held at the University of Leeds

Zaefarian F, Rezvani M, Ardakani MR, Rejali F, Miransari M (2013) Impact of mycorrhizae formation on the phosphorus and heavy-metal uptake of *Alfalfa*. Commun Soil Sci Plant Anal 44:1340–1352. https://doi.org/10.1080/00103624.2012.756505

Chapter 2
Dynamics of Arbuscular Mycorrhizal Symbiosis and Its Role in Nutrient Acquisition: An Overview

Purnima Bhandari and Neera Garg

Abstract Arbuscular mycorrhiza constitute a heterogeneous group of diverse fungal taxa that have been reported to form mutualistic interaction with the roots of more than 90% of all plant species. Accomplishment of this symbiotic interaction requires a high degree of synchronization between the two partners and is based on a finely regulated molecular dialogue. Where plant roots exude strigolactones that stimulate fungal metabolism and branching, fungus releases signaling molecules—myc factors that trigger symbiotic responses in the host plant. Among the various benefits bestowed by this symbiotic association, transport of limiting soil nutrients including phosphorus (P), nitrogen (N), sulphur (S) in exchange for fixed carbon is considered as the key feature which occurs in arbuscule containing host cortical cells. In the last few years, novel transporters involved in this mutualistic interaction have been unravelled. This chapter briefly summarizes the signaling pathways and nutrient exchange involved in the establishment of an effective symbiosis between the host plant and fungus that could provide better insight into the role of mycorrhizal fungi in sustainable agriculture.

2.1 Introduction

In the soil rhizosphere, plant roots interact with a number of beneficial microorganisms, among which arbuscular mycorrhizal (AM) fungi are recognized as one of the most significant group of soil biota in the context of ecosystem sustainability (Jeffries and Barea 2012, Barea et al. 2013). AM fungi, belonging to the phylum Glomeromycota have been documented to form symbiosis with more than 90% of plant species belonging to Angiosperms, Gymnosperms and Pteridophytes (Read et al. 2000; Shah 2014; Prasad et al. 2017). It is an ancient type of interaction that have been believed to facilitate colonization of land even more than 460 million years ago (Redeker et al. 2000; Smith and Read 2008). Due to their widespread

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

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_2

occurrence, they are considered as 'ecosystem engineers' of plant communities (Cameron 2010; Bücking and Kafle 2015) as they affect the distribution and movement of nutrients within the soil ecosystem through the activities of the interlinked and extensive soil extra-radical mycelium (ERM) (Richardson et al. 2009; Barea et al. 2014). The major flux is the transfer of carbon (C) from the host plant to fungus (and thereby to the soil) and the reciprocal movement of phosphate and ammonium (NH_4^+) from fungus to plant (Barea et al. 2014). This mutualistic interaction results in the formation of tree-shaped subcellular structures within cortical cells of plant roots called 'arbuscules' (derived from the Latin arbusculum, meaning bush or little tree), thus establishing main interface for symbiotic nutrient transfer (Parniske 2008; Gutjahr and Parniske 2013; López-Ráez and Pozo 2013). However, rather than developing on a leaf, AM interaction occurs beneath the soil surface, thus strongly hindering chances of understanding most of the early steps of their development including signal exchange that occurs between plant and the endophytic fungus (Genre 2012). Following sub sections briefly summarize the (1) events that lead to the establishment of AM symbiosis in plant rhizosphere and, (2) recent advancements in nutrient exchange and metabolite fluxes among the two symbionts.

2.1.1 AM Establishment: An Overview

AM symbiosis, which have been reviewed recently by various authors (Garg and Chandel 2010; Genre 2012; Aroca et al. 2013; Gutjahr and Parniske 2013; Barea et al. 2014; Bonfante and Desirò 2015; Mohanta and Bae 2015) is a complex and very dynamic interaction that requires a high degree of coordination between the two partners and is based on a finely regulated molecular dialogue (Hause et al. 2007; López-Ráez et al. 2010; Aroca et al. 2013; Barea et al. 2014; Pozo et al. 2015; López-Ráez 2016). The establishment of AM symbiosis can be divided into three different growth stages: (1) asymbiotic hyphal growth stage, where spores germinate and develop hyphae autonomously but for a limited period; (2) pre-symbiotic growth stage, where hyphal growth is stimulated by host signal perception; and (3) symbiotic stage, in which fungus penetrates plant root and develops both intraradical mycelium (IRM, to exchange nutrients) as well as extra-radical (ER) hyphae (to recruit nutrients in the soil and form new spores; Smith and Read 2008; López-Ráez and Pozo 2013).

Generally, it has been validated that AM fungi colonize plant roots from three main types of soil-based propagules: spores, fragments of mycorrhizal roots and ER hyphae, all of them producing more or less a well-developed mycelial network expanding in the soil (Barea et al. 2014). During the first phase, AM fungal colonization initiates with the formation of hyphae that arises from soil-borne propagules i.e. resting spores or mycorrhizal root fragments or from AM plants growing in the vicinity (Koltai and Kapulnik 2009). Following germination, fungus uses triacylglyceride (TAG) and glycogen reserves in the spore to support growth of

such a short mycelium as it is unable to uptake C from the soil organic matter (Harrison 2005; Leigh et al. 2009). However, in the absence of host (i.e. during asymbiotic phase), these germinating hyphae can grow only for a few days. Due to their obligate biotrophic nature and short life span, growth of such asymbiotic hyphae ceases before the spore reserves are depleted; as a result, in view of new germination event, mycelium retract their cytoplasm into spore (Genre 2012) and thus, return to the dormant stage.

Such exploratory hyphal development pattern changes dramatically once the hyphae reach the vicinity of a host root (pre-symbiotic growth phase) and respond to their proximity (Balestrini and Lanfranco 2006; Nasim 2013). As a result, the growth of hyphal germ tube increases substantially and hyphae ramifies intensively through the soil towards the host root (López-Ráez et al. 2012) which suggests that they have perceived something exuded from the root (Harrison 2005). Plant roots release a wide range of compounds, among which *strigolactones* (SL) have been recognized as an important 'rhizospheric plant signals' involved in stimulating the pre-symbiotic growth of AM fungi at different stages, i.e. during spore germination stage and during hyphal growth and branching stage (Fig. 2.1b; Akiyama et al. 2005; Gómez-Roldán et al. 2008; López-Ráez et al. 2012) that enhance the chances of an encounter with the host (Kumar et al. 2015), thus causing successful root colonization by AM fungi. Various studies have validated the relevance of SL in the establishment of AM symbiosis where reduction in the process of mycorrhizal colonization of mutant plants have been observed due to the impairment in SL biosynthesis (Gómez-Roldán et al. 2008; Vogel et al. 2010; Kohlen et al. 2012; López-Ráez and Pozo 2013). SL are present in extremely low concentrations in the root exudates (Akiyama and Hayashi 2006) and their concentration tends to increase under the sub-optimal growth conditions such as limited nutrition, etc., that favour mycorrhizal colonization (Yoneyama et al. 2007; Koltai and Kapulnik 2009). Yoneyama et al. (2012) revealed that biosynthesis and exudation of SL gets boosted under phosphate starvation, a condition that promotes AM colonization. Various studies have authenticated that only the molecules released from the host plant are perceived by the fungus (through a so far uncharacterized receptor) that stimulate hyphal branching in AM fungi, indicating that discrimination between host and non-host occurs at this stage (Harrison 2005; Nasim 2013). These findings clearly signify that the fungus possesses mechanism to perceive active root molecules and to switch on specific transcriptional pathways which induces morphological changes in the fungus and activate its growth (Balestrini and Lanfranco 2006; Nasim 2013). Thus, extensive hyphal branching of AM fungi, as induced under the influence of SL maximizes the chance of contact with host root and that of establishing symbiosis (Akiyama et al. 2005; Aroca et al. 2013; López-Ráez and Pozo 2013). Conversely, when AM fungus starts to proliferate in the vicinity of the root, plants perceive diffusible fungal signals, called *Myc factors* at the plant plasma membrane, due to lysine-motif (LysM) receptor kinases (Antolın-Llovera et al. 2012; Oldroyd 2013) that actively prepares the intracellular environment and induce symbiosis-specific responses in the host root, even in the absence of any physical contact (Parniske 2008; Genre and Bonfante 2010). The chemical structure

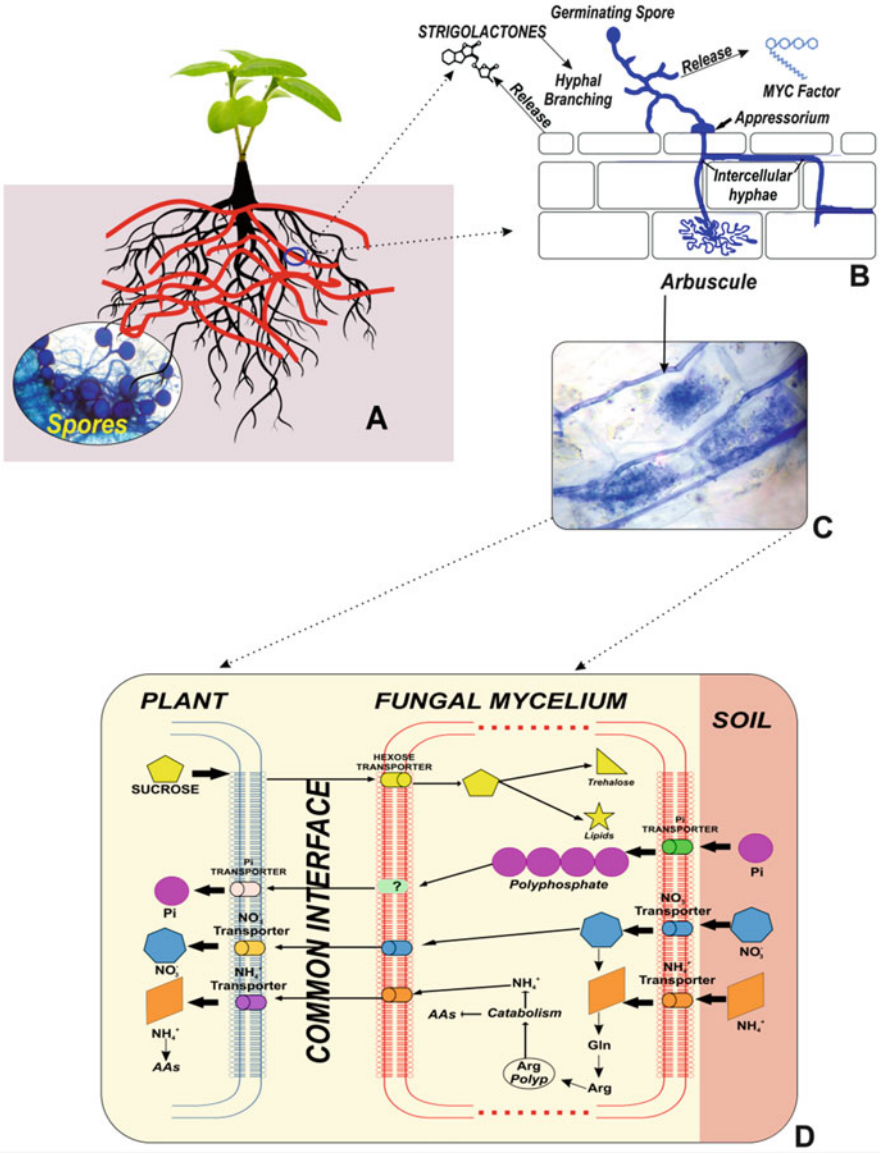


Fig. 2.1 The scheme illustrates (**a**) plant colonized with AM fungi and fungal spores (**b**) Different steps of AM establishment: Roots of host plant exude *strigolactones* which induce spore germination and hyphal branching. In response, fungus releases *Myc* factors that induce calcium oscillations in root epidermal cells and activate plant *SYM* genes, thus leads to the formation of *hyphopodium*, consequently *arbuscule*. (**c**) Arbuscule formation and (**d**) Bidirectional transfer of nutrients (C, P, N) at plant-fungus interface as well as AM fungus-soil interface: At the plant-fungus interface, carbon (C) is imported from plant *via* hexose transporters to the fungal mycelium where it is stored either in the form of trehalose, glycogen and lipids. In return, fungus helps in acquisition of mineral nutrients including phosphorus (P), nitrogen (N). Different forms of N such

of these elusive Myc factors was identified as a mixture of sulphated and non-sulphated lipochito-oligosaccharides (Myc-LCO) that shares structural similarities with rhizobial Nod factors (Maillet et al. 2011). In addition to the up-regulation of genes involved in signal transduction, Myc factors are also known to activate a number of plant responses including stimulation of lateral root development, starch accumulation and repeated calcium oscillation in epidermal cells, in analogy with *Rhizobium*-legume symbiosis (Kosuta et al. 2008; Gutjahr et al. 2009; Maillet et al. 2011; Gutjahr and Parniske 2013; Bonfante and Desirò 2015).

Pre-symbiotic phase is trailed by direct plant–fungus contact i.e. symbiotic phase, which results in the setup of novel developmental and cellular modifications in both partners. Perception of Myc factor induces nuclear Ca^{2+}-spiking that is decoded by a nuclear localized calcium-calmodulin kinase (CCaMK) and leads to phosphorylation of transcription factor—CYCLOPS which in turn cause transcriptional activation of symbiosis-related genes (Genre et al. 2013; Singh et al. 2014; Gutjahr 2014; Carbonnel and Gutjahr 2014). The hyphae of glomeromycetes adheres to atrichoblasts of the root epidermis by forming a highly branched, swollen and flattened characteristic fungal structure called *appressorium* (also called hyphopodium) (Fig. 2.1b; Smith and Read 2008; Genre 2012) which marks the initiation of symbiotic phase of the interaction. Accordingly, root epidermal cells respond to this appressorium formation by repositioning their nucleus and re-modelling their cytoplasm, thus preparing themselves for fungal penetration (Genre et al. 2005). A proline rich protein which is encoded by ENOD11 activates the epidermal cells before, during the formation of pre-penetration apparatus (PPA) and at the late stage of mycorrhizal development and even in the arbuscule-containing cells (Mohanta and Bae 2015). From the appressorium, a penetrating hypha is formed, which reaches the root cortex by following an intracellular route across epidermal cells (Barea et al. 2014). Host cell integrity is maintained by the invagination of its plasma membrane (i.e. peri-arbuscular membrane—PAM) which proliferates and engulfs the developing hypha, physically separating fungus from the plant cytoplasm (Bonfante and Desirò 2015), there by guiding intracellular fungal passage into deeper cortical layers. For this step to be successful, three ion channel genes of *M. truncatula* DMI1 (does not make infection 1), DMI2, and DMI3 are considered essential for the induction of PPA in plants (Siciliano et al. 2007; Genre and Bonfante 2010; Mohanta and Bae 2015). In the inner cortical cells, fungal hyphae ramify repeatedly in order to differentiate into *arbuscules*—the small characteristic tree like structures (Fig. 2.1c) which represents symbiotic interface

Fig. 2.1 (continued) as inorganic as well as organic forms (NH_4^+, NO_3^-) are taken up by specialized transporters located on the fungal membrane in the extraradical mycelium (ERM) where they imported from the symbiotic interface to the host plant cells *via* selective transporters. Pi is taken into fungal ERM through Pi transporters and is converted into polyP which is transported from ERM to IRM where polyp hydrolysis occur, thus releases Pi in the IRM. From IRM, plant take up Pi *via* Pi transporter present at mycorrhizal plasma membrane

where nutrient exchange between fungus and plant is thought to occur (Smith and Read 2008). The proteins encoded by common symbiosis (SYM) genes (CCaMK and CYCLOPS) are also involved in the intracellular fungal accommodation: mutants for SYM genes are not only defective in the signalling pathway (as testified by the lack of nuclear calcium spiking), but also in the assembly of the PPA, thus subsequent fungal colonization (Bonfante and Genre 2010; Genre 2012). In addition, *VAPYRIN* is another gene, which has been reported to be potentially involved in the structural reorganization of infected cells (Gutjahr and Parniske 2013). In case of vapyrin mutants of *Medicago* and *Petunia*, rhizodermal penetration is frequently aborted and in rare cases, where cortical colonization is achieved, no arbuscules or only small hyphal protrusions into cortical cells have been observed (Reddy et al. 2007; Pumplin et al. 2010; Gutjahr and Parniske 2013). Appearance of arbuscule inside the lumen of inner cortical cells hallmarks the establishment of symbiosis between the two partners (Genre 2012). On the basis of morphological attributes, two types of AM associations have been reported which include Arum type colonization and Paris type colonization (Garg and Chandel 2010). In the Arum type, which is particularly common in legumes, and in general in those plants whose root anatomy presents extensive apoplastic channels (Genre 2012), AM fungi form extensive intercellular hyphae in well-developed air spaces between cortical cells and invaginate cells as short side branches to form arbuscules (Shah 2014). Contrastingly, in the Paris type, colonization spreads directly from cell to cell in the root and is characterized by the absence of intercellular hyphae and the development of intracellular hyphal coils that frequently have intercalary arbuscules (Shah 2014). In addition to arbuscules, some fungi also form lipid containing storage structures known as vesicles in the root apoplast (Walker 1995; Rouphael et al. 2015).

As IRM grows, a dense net of ERM is formed simultaneously in the soil (Malbreil et al. 2014) which helps in the acquisition of mineral nutrients from the strata, particularly those nutrients whose ionic forms have poor mobility or are present in low concentration in the soil solution, such as phosphate and ammonia (Barea et al. 2005, 2014). In addition, ERM interacts with other soil micro-organisms and colonizes the root of adjacent plants belonging to the same or different species. Thus, plants and their AM fungi are interconnected through a web of roots and hyphae (Read 1998; Giovannetti et al. 2004) where exchange of water, nutrients as well as signals (Song et al. 2010) occur (Rouphael et al. 2015). Finally, ERM forms new chlamydospores and helps in the propagation of fungus, thus completing the lifecycle. Therefore, establishment of a functional AM symbiosis involves a high degree of coordination between plant and fungus, as characterized by progressive increase in the closeness of the interaction, from the exchange of long-range chemical signals in the rhizosphere to intimate intracellular association, where plant and fungus share a single cell volume (Genre 2012).

2.1.2 AM Fungi Improve Nutrient Dynamics in the Rhizosphere

Nutrient exchange is a key function that takes place at the symbiotic interface, formed in association between the roots of AM fungi and their host plants (Jakobsen and Hammer 2015) and is bidirectional in nature (Fig 2.1d). However, for many of these fungi, the specific mechanisms and gene products involved in nutrient transfer remain to be elucidated (Behie and Bidochka 2014). AM fungi are obligate biotrophs and are unable to absorb carbohydrates; as a result, they depend totally on their green host for organic C metabolism (Bonfante and Desirò 2015). In return, by acting as an extension of root system, thus increasing the plant surface area for absorption, ERM of the fungus provides host plant with access to nutrient resources such as phosphorus (P), nitrogen (N), sulphur (S) and various trace elements beyond the root depletion zone through the agency of IRM, where nutrients are exchanged at the fungus-plant interface for the fixed C (Fig. 2.1d; Marschner and Dell 1994; Smith et al. 2009; Fellbaum et al. 2014; Jakobsen and Hammer 2015). Studies have estimated that host plant transfers up to 20% of its photosynthetically fixed C to the AM fungus (Wright et al. 1998; Valentine et al. 2013; Fellbaum et al. 2014; Bücking and Kafle 2015) which is used to maintain and extend its hyphal network in the soil. However, maintenance of such cooperation has posed a paradox for evolutionary theory as it is hard to explain such kind of interaction where selfish individuals can exploit mutualisms, reaping benefits while paying no costs (Leigh 2010; Fellbaum et al. 2014). Recent studies have revealed that the flow of C to the fungus can be downregulated under sufficient nutrient regimes and that the fungus is also able to control transfer of nutrients to less than beneficial host (Kiers et al. 2011; Maillet et al. 2011; Valentine et al. 2013). Thus, it has been suggested that C to nutrient exchange in mycorrhizal symbiosis is controlled by biological market dynamics and that reciprocal reward mechanisms ensure a 'fair trade' between both the partners involved in AM symbiosis (Kiers et al. 2011; Bücking and Kafle 2015). AM fungi are able to absorb different macro-as well as -micronutrients; however, the likeliness for P uptake is higher when compared with the uptake of other nutrients which could be credited to the production of some enzymes such as phosphatase by the fungi, that enhances the solubility of insoluble P and hence it's absorption by plant (Smith and Read 2008). However, when the concentration of nutrients is higher in the rhizosphere, symbiotic efficiency usually decreases which is due to the presence of nutrient receptors in plant cellular membrane that gets adversely affected (Miransari 2013). Thus, under low and medium nutrient concentrations, the dependency of the host on glomeromycotan fungi increases (Smith and Read 1997; Valentine et al. 2013).

2.1.2.1 Phosphorus Metabolism

P is an important macronutrient that is required for energy pathways and production in plant as well as for the structure of proteins and production of cellular membranes

(Miransari 2013, Bakshi et al. 2017). It is preferentially taken up as orthophosphate (Pi) by plants, but unfortunately, this form occurs at low concentrations in soils, around 10 mM (Bieleski 1973) due to its low solubility and low mobility, leading to a rapid depletion zone around the roots (Malbreil et al. 2014). Improved uptake of P is the main benefit that plants obtain by associating with AM fungi which has been validated through the use of $^{32}P/^{33}P$-based isotope dilution approaches (Barea 2010) and has led to the conclusion that the majority of P taken up by plants comes via the fungal partner (Smith et al. 2009; Smith and Smith 2011, 2012; Barea et al. 2014). In general, plant uses two different pathways to absorb P from the soil: the direct uptake by plant roots (i.e. plant uptake pathway, PP) and the indirect uptake by glomeromycotan fungi (i.e. mycorrhizal pathway, MP). Two different phosphate transporters (PTs) are activated during the uptake of P including the ones which are located in the epidermis and root hairs (i.e. PP) and the fungal hyphal transporters, which are localized with a few centimeters in distance from the plant roots (i.e. MP) (Bücking et al. 2012; Miransari 2013). Due to much smaller diameter than roots, the individual fungal hyphae allow access to narrower soil pores and hence enhance the soil volume explored (Drew et al. 2003; Smith and Read 2008; Smith et al. 2011). The active Pi are taken up into the ERM against a large electrochemical potential gradient, high-affinity PTs and energized by H^+-ATPases (Harrison and van Buuren 1995; Ferrol et al. 2000; Bucher 2007; Javot et al. 2007ab; Smith and Read 2008; Smith and Smith 2011). P, thus absorbed by the fungal hyphae, is then translocated as polyphosphate (polyp, Fig. 2.1d) to the specialized AM fungal-plant interfaces i.e. arbuscules and hyphal coils (Smith et al. 2011) where it gets hydrolysed to free Pi that gets delivered in the apoplast, from where a specialized host plant transporter takes care of importation (Malbreil et al. 2014). Different studies have revealed that mycorrhizal pathway can deliver up to 100% of plant P uptake (Ravnskov and Jakobsen 1995; Smith et al. 2003) thus indicating that the uptake at the root epidermis is very low either due to down-regulation of direct plant PTs (Javot et al. 2007b; Yang et al. 2009; Grønlund et al. 2013) or due to the reduced Pi concentration in the rhizosphere soil solution (Jakobsen and Hammer 2015).

A major breakthrough in mycorrhizal symbiosis was achieved when PT gene was characterized from the ER hyphae of *Glomus versiforme* (GvPT), involved in Pi uptake from soil (Harrison and van Buuren 1995; Smith and Smith 2011; Mohanta and Bae 2015). This Pi gene was induced at the transcriptional level in the presence of lower amount of Pi. Later on, another PT homolog (GmosPT) showing a similar role in Pi transport was reported from *Funneliformis mosseae* (formerly *Glomus mosseae*; Benedetto et al. 2005). Interestingly, a relatively high expression level of the transcript, independent of external Pi concentrations was observed in IR fungal structures (Benedetto et al. 2005) suggesting that the fungus may exert control over the amount of phosphate delivered to the plant inside the root cell (Balestrini and Lanfranco 2006). In addition to PTs, genes encoding alkaline phosphatases have been expressed in *R. irregularis* (formerly *Glomus intraradices*) and *G. margarita* (Tisserant et al. 1993; Aono et al. 2004). In such cases, levels of the corresponding transcripts were found to be higher in

mycorrhizal roots than in germinating spores and external hyphae, thus advocating their role in nutrient exchange with host plants (Aono et al. 2004; Mohanta and Bae 2015). Conversely, on the plant side, PTs operating at the root–soil interface have been reported to be downregulated. As a result, host plant largely depends on the phosphate delivered by the fungal symbiont (Smith et al. 2003). Various studies have highlighted the presence of PTs which are exclusively expressed during symbiosis (Harrison et al. 2002; Paszkowski et al. 2002; Karandashov and Bucher 2005; Balestrini and Lanfranco 2006). In case of *M. truncatula*, plant PT—MtPT4 was found to be located at PAM, where it likely plays an essential role in phosphate transport into the cell (Harrison et al. 2002). However, loss of MtPT4 function led to the premature death of arbuscules in *M. truncatula* plant and fungus was unable to proliferate within the host root and consequently, resulted in the termination of symbiosis (Javot et al. 2007a; Mohanta and Bae 2015). Thus, it could be established that in addition to increase in plant Pi acquisition, mycorrhizal-induced PTs play an important role in maintaining symbiosis by regulating arbuscule morphogenesis (Javot et al. 2011; Yang et al. 2012; Xie et al., 2013; Berruti et al. 2016). At present, accumulating evidence confirms that AM symbiosis specifically induces the expression of plant PTs (Harrison et al. 2002; Paszkowski et al. 2002; Nagy et al. 2005; Xie et al. 2013; Walder et al. 2015; Berruti et al. 2016). These genes include OsPT11 (*Orzya sativa* phosphate transporter11), LePT4 (*Lycopersicon esculentum* PT4), PtPT8 (*Populus trichocarpa* PT8), PtPT10 (*P. trichocarpa* PT10), StPT4 (*Solanum tuberosum* PT4), StPT5 (*S. tuberosum* PT5), LePT4 (*L. esculentum* PT4), PhPT4 (*Petunia hybrid* PT4), PhPT5 (*P. hybrid* PT5), LjPT3 (*Lotus japonicus* PT3), GmPT7 (*G. max* PT7), GmPT11 (*G. max* PT11), GmPT10 (*G. max* PT10), ZmPT6 (*Zea mays* PT6) (as reviewed by Berruti et al. 2016). Recently, Volpe et al. (2016) studied the expression of AM-induced Pi transporters in *M. truncatula* (MtPT4) and *L. japonicus* (LjPT4) and found their expression in the root tips of even non-colonized plants, thereby postulating PT4 genes as novel component of Pi-sensing machinery in the root tips. However, it has been observed that the amount and availability of P in the soil greatly affects its uptake. Under higher P availability, AM fungi may not be able to efficiently colonize host plant roots (due to decreasing arbuscule development), because under such conditions, host plant may not be willing to spend energy for the development of symbiotic association (Miransari 2013). Conversely, under P deficit conditions, fungus is able to colonize the roots of host plant efficiently, thus significantly enhances P uptake by the host plant (Smith and Read 2008).

2.1.2.2 Nitrogen Metabolism

In addition to P, fungal partner also improves the performance of plant partner by providing N nutrient from both inorganic and organic N sources (Hodge et al. 2001; Leigh et al. 2009; Hodge and Fitter 2010; Matsumura et al. 2013; Kranabetter 2014; Corrêa et al. 2015; Mohanta and Bae 2015). In almost all ecosystems, availability of N limits primary productivity (Behie and Bidochka 2014). N bounded in the

organic matter is typically present in the form of peptides, proteins and free amino acids (FAA). AM fungi release peptidases and proteases into the soil that cleave organically bound N and subsequently absorb nitrogenous monomers (Nygren et al. 2007; Behie and Bidochka 2014). According to McFarland et al. (2010), mycorrhizal fungi are able to cater 50% of plant N requirement. When compared with P, N is a more mobile nutrient, hence its uptake by mycorrhizal plant may be of less importance, as N can be supplied to the host plant through mechanisms such as diffusion and mass flow (Miransari 2013). The ability of mycorrhizal fungi to utilize mineral N from organic matter and amino acids has been indicated through different studies (St. John et al. 1983; Hodge et al. 2001; Hamel 2004). However, various factors such as volume of fungal network, amount of decomposing (hydrolytic) enzymes such as xyloglucanase, pectinase, interaction with other soil microbes may affect the ability of fungus to mineralize higher amounts of organic N (Miransari 2013). In addition, by providing P, mycorrhizal fungi also improve N_2-fixation, thus representing a considerable contribution to N inputs in legume species (Azcón and Barea 2010; Barea et al. 2014).

AM fungi have been reported to directly take up and transfer N to their host plants (Bago et al. 1996; Johansen et al. 1993; He et al. 2003), thereby enhancing the utilization of different forms of N such as nitrate (NO_3^-), ammonia (NH_4^+) and urea to plants (Hodge et al. 2001). They easily translocate such different forms of N from ERM (incorporated into amino acids) to the IRM mainly as arginine via respective transporter molecule, where arginine (transported in association with polyP) would be broken down through urease cycle into NH_4^+ and thus, N is transferred to the plant without any C skeleton (Balestrini and Lanfranco 2006; Pérez-Tienda et al. 2011; Malbreil et al. 2014; Mohanta and Bae 2015). This hypothetical pathway was validated by the work of Tian et al. (2010) who demonstrated that during fungal association, arginine in roots of host plant increased threefold and was found to be the most abundant FAA owing to the presence of fungus inside the root. However, the molecular form in which N is transferred, as well as the involved mechanism is still under debate (Mohanta and Bae 2015). NO_3^- is the dominant form of N that is available to plants and fungi in most of the agricultural soils, while NH_4^+ predominates in many undisturbed or very acidic soils, where NO_3^- can be almost entirely absent (Bücking and Kafle 2015). The ERM of AM fungi can take up NH_4^+ (Frey and Schüepp, 1993) and NO_3^- (Hawkins et al. 2000), but NH_4^+ is generally preferred, because it is energetically more efficient than NO_3^-. Moreover, NH_4^+ seems to be the preferred molecule (Guether et al. 2009) as upon root colonization with *G. margarita*, the transcript of LjAMT2 (NH_4^+ transporter) was found to be up-regulated in transcriptome analysis of *L. japonicus*. Moreover, this transcript was found to be extensively expressed in mycorrhizal root, but not in the nodule (Guether et al. 2009). Recently, various transcriptome studies have revealed the expression of several fungal NH_4^+ and NO_3^- transporters in spores, ERM and IRM (Tisserant et al. 2012). Two high-affinity N transporters have been partially characterized in *R. irregularis* where the expression of GintAMT1, an NH_4^+ transporter was induced by low additions of NH_4^+ to the medium but was found to be suppressed under high NH_4^+ supply, thus suggesting

that the expression of this transporter is substrate inducible and is regulated by NH_4^+ supply as well as by fungal NH_4^+ status (López-Pedrosa et al., 2006; Bücking and Kafle 2015). However, under N limiting conditions, NH_4^+ transporter GintAMT2 was found to be constitutively expressed in the ERM (Pérez-Tienda et al. 2011). Thus, such differential localization of high transcript levels of these transporters in colonized roots suggest that both transporters may differ in their role for N uptake and transport (Bücking and Kafle 2015). High expression levels of GintAMT1 in the ERM suggest that this transporter could be primarily involved in NH_4^+acquisition of fungal hyphae from the soil, while, higher expression of GintAMT2 in the IRM signifies the role of this transporter in the re-uptake of NH_4^+ by the fungus from the symbiotic interface (Pérez-Tienda et al. 2011). The ability to transfer N has also been explored in other *Glomus* species, such as in *F. mosseae*, where AMT (GmAMT4.1) was identified during arbuscule development inside roots of *G. max* (López-Pedrosa et al. 2006; Behie and Bidochka 2014). Similarly, in case of *Medicago* mutants, it was demonstrated that in addition to PT, AMT symbiotic transporters (i.e., PT4 and AMT2; 3) did had an influence on the arbuscule lifespan (Javot et al. 2007b; Breuillin-Sessoms et al. 2015), thus speculating that the transport of Pi or NH_4^+ through these transporters not only deliver nutrients to the host root cells but also trigger signalling that enable the conditions for arbuscule maintenance (Breuillin-Sessoms et al. 2015; Berruti et al. 2016). To be further assimilated *via* glutamine synthetase/glutamate synthase (GS/GOGAT) cycle (Marzluf 1996), NO_3^- has to be converted into NH_4^+ by the sequential action of enzymes—nitrate reductase (NR) and nitrite reductase (NiR). One transcript for NR and two for NiR were identified in *R. irregularis*, all of which got expressed in ERM (Malbreil et al. 2014). Furthermore, transcripts coding for proteins that are involved in further steps to synthesize arginine were identified and were found to be highly expressed in germinating spores, ERM and IRM, thus confirming intense N cycling in this fungus (Tian et al. 2010; Tisserant et al. 2012).

In addition, plants that accommodate fungal translocation of N were found to upregulate N transporters. Plant NH_4^+ transporters were found to be upregulated in arbuscule-containing cells in case of sorghum (*S. bicolor*), where expression of plant NH_4^+ transporters—SbAMT3; 1 and SbAMT4 was induced only in arbuscule-containing cells (Koegel et al. 2013). Similarly, in case of *M. truncatula*, NO_3^- transporters were expressed in arbusculated cells (Gaude et al. 2012; Behie and Bidochka 2014). Such coordinated and specific expression of both plant and fungal $NH_4^+$and NO_3^- transporters in mycorrhizal-colonized cortical cells intimate the crucial importance of fungal N transfer in plants (Behie and Bidochka 2014).

2.1.2.3 Sugar Metabolism

Carbon flux is mainly mediated from plant to the fungus as mycorrhizal fungi are incapable of breaking down complex organic compounds (Behie and Bidochka 2014) and thus, depends upon the host plant for C. Mycorrhizal fungi require C for extension of ERM, for active uptake or other energy consuming processes and for

the development of new infection units (Bücking et al. 2012). Moreover, it has been validated that supply of C by the host plant stimulates P uptake and its transfer by AM fungi (Kiers et al. 2011; Hammer et al. 2011; Bücking et al. 2012). Earlier, it was shown that C is mainly delivered by the host plant in the form of hexoses, preferentially as glucose (Shachar-Hill et al. 1995; Solaiman and Saito 1997; Pfeffer et al. 1999; Malbreil et al. 2014) or in the form of sucrose into the apoplast, where it is converted into hexoses by acid invertase, secreted by host plant (Schaarschmidt et al. 2006) as fungus lacks the ability to secrete this enzyme (Tisserant et al. 2013). Hexoses are then transferred to the mycorrhizal fungi *via* fungal transporters that function at several symbiotic root locations (Schüβler et al. 2006; Helber et al. 2011; Mohanta and Bae 2015). In *Glomus* species, a high-affinity monosaccharide transporter (MST)—MST2 has been characterized by Bücking and Shachar-Hill (2005) whose expression pattern correlates with that of the mycorrhizal PT–PT4. Various studies have revealed that when expression of PT4 is reduced, symbiosis gets strongly impaired, resulting in malformed arbuscules, however, when incorporated, hexoses are then converted into trehalose, glycogen and lipids (Shachar-Hill et al. 1995; Pfeffer et al. 1999; Bago et al. 2000), thus signifying the fact that the amount of C received by the fungal symbiont is directly interrelated to the phosphate transfer efficiency. Moreover, many MST have been isolated and identified from different fungal species such as one MST from *G. pyriformis* (Schüβler et al. 2006), 3 MSTs as well as a sucrose transporter from *R. irregularis* (Helber et al. 2011). Triacylglycerol (TAG) is the main form of C stored by the mycobiont at all stages of its life cycle (Bago et al. 2003) which is mostly or exclusively made in IRM and gets transferred to ERM (Pfeffer et al. 1999). In vivo microscopic observations suggests that the rate of export is sufficient to account for the high levels of stored lipid in ERM (Bago et al. 2002, 2003) where glyoxylate cycle operates (Lammers et al. 2001) and converts exported TAG to carbohydrate. Trehalose and glycogen synthases were found to be present in the transcript collection of *R. irregularis* by the authors (Tisserant et al. 2012, 2013). C, thus formed in ERM, is finally used for the production of the chitinous cell wall (Lanfranco et al. 1999), for storing lipids and glycogen in the developing spores (Bonfante et al. 1994) and for long-lasting proteins like glomalin (Purin and Rillig 2007).

During the symbiotic phase, C metabolism of both the symbiotic partners get reformed at the level of gene expression (Balestrini and Lanfranco 2006). In AM-colonized roots, sucrose synthase gene was found to be up-regulated by Ravnskov et al. (2003) suggesting that the enzyme sucrose synthase plays a major role in generating sink strength (Mohanta and Bae 2015). In addition, mycorrhizal colonization has been reported to elevate the expression levels of plant sugar transporters. For instance, in the symbiotic interaction between *M. truncatula* and *R. irregularis*, increased expression of MtSucS1, a plant sucrose synthase gene, has been recorded in the surrounding internal hyphae and arbuscules (Behie et al. 2012). Besides, the expression of a family of *M. truncatula* sucrose transporters—MtSUTs increased in mycorrhizal-colonized roots. Currently, it is not possible to unravel which partner takes the first step to establish the mutualistic

C–P exchange (Smith and Smith 2012; Jakobsen and Hammer 2015). However, it has been proposed that fungus might be able to use plant cell wall sugars (Helber et al. 2011), while P reserves in spore of AM fungi could serve as signals during early colonization (Hammer et al. 2011).

2.1.2.4 Sulphur Metabolism

Sulphur (S) is an essential macronutrient required for plant growth, development and response to various abiotic and biotic stresses as it plays as key role in the biosynthesis of many S-containing compounds. Sulphate represent a very small portion of soil S pull and is the only form that plant roots can uptake and mobilize through H^+-dependent co-transport processes, thereby implying the role of sulphate transporters (Casieri et al. 2012; Miransari 2013). In contrast to the other organically bound forms of S, sulphate is commonly leached from soils due to its solubility in water, thus reducing its availability to plants (Eriksen and Askegaard 2000; Casieri et al. 2012). During mycorrhizal interactions, by altering the expression of plant sulphate transporters (Casieri et al. 2012; Giovannetti et al. 2014), fungal symbiont plays an important role in the uptake of S, thereby improving S nutritional status of the host plant (Allen and Shachar-Hill 2009; Casieri et al. 2012; Sieh et al. 2013; Berruti et al. 2016). In order to understand the beneficial role of mycorrhizal interaction on *M. truncatula* plants colonized with *R. irregularis* at different sulphate concentrations, Casieri et al. (2012) analyzed the expression of genes encoding putative *Medicago* sulphate transporters (MtSULTRs) and revealed that mycorrhizal symbiosis substantially increased the rate of plant S absorption. Moreover, in silico analyses they identified and recognized eight MtSULTRs, some of which were expressed in plant leaf and root at different S concentrations, thus demonstrated the role of AM fungi on S uptake by the host plant. Recently, a sulphate transporter (A group 1 sulfate transporter, LjSultr1; 2) specifically involved in the uptake of S from arbuscules has been identified in *L. japonicus* (Giovannetti et al. 2014). However, in contrast to PTs, a single gene LjSultr1; 2, seems to mediate both direct and symbiotic pathways of S uptake in *L. japonicus*. On the contrary, the efficiency of S uptake in plant roots was directly correlated with phosphate availability, as transfer of S increased only when the phosphate content of the soil was low (Sieh et al. 2013; Behie and Bidochka 2014). In addition, the effects of mycorrhizal colonization on the S uptake by the host plant could also be explained on the basis of higher production of root exudates, increased activity of other soil microbes such as *Thiobacillus*, formation of extensive hyphal network and production of different enzymes, which may acidify the rhizosphere and hence increase the availability of S to the host plant (Miransari 2013).

2.1.2.5 Other Macro-as Well as Micro-nutrient Metabolism

Apart from P, N and S, mycorrhizal fungi are able to increase the uptake of different macro-as well as-micro nutrients including potassium (K), magnesium (Mg),

calcium (Ca), zinc (Zn), copper (Cu) and iron (Fe) under various environmental conditions. AM fungi develop an extensive network of hyphae that reaches into the even finest soil pores producing different enzymes such as phosphatases, which enhances the solubility of nutrients and hence their subsequent uptake by the host plant (Miransari 2013). In addition, enhanced uptake of water and plant growth, with a larger root medium, as observed under mycorrhization substantially increases the rate of nutrient uptake (Smith and Read 2008). In a split-plot experiment performed under field conditions, mycorrhizal fungi *R. irregularis* improved the uptake of different nutrients including K, Mg and Ca in tomato (*L. esculentum*; Cimen et al. 2010; Miransari 2013). Furthermore, several authors have reported up-regulation of a plant K^+ transporter in mycorrhizal roots of *L. japonicus* (Guether et al. 2009; Berruti et al. 2016).

In case of micronutrients, AM fungi help plant in two ways: (1) they help in the uptake of these elements which are considered to be relatively immobile, and (2) take up these elements and store them so as to prevent their concentrations to reach toxic levels (Goltapeh et al. 2008). AM fungi mobilize such micronutrients either by producing different enzymes or by interacting positively with the other soil microbes or by modifying plant rhizosphere or by affecting the morphology (i.e. root growth) and physiology of the host plant, thus affecting the production of root exudates (Miransari 2013). In one of the studies, Zaefarian et al. (2011) demonstrated the beneficial effects of different fungal species including *F. mosseae*, *G. etunicatum* and *R. irregularis* (as single treatments) and the combined treatment of *F. mosseae*, *Gigaspora hartiga* and *G. fasciculatum* on the uptake of N, P, K, Fe, Zn and Cu. In addition, two meta-analysis studies have been published recently, focusing on the contribution of mycorrhizal symbiosis to different micro-nutrient concentrations in crops (Lehmann et al. 2014; Lehmann and Rillig 2015; as reviewed by Berruti et al. 2016). According to Lehmann et al. (2014), factors such as soil texture, pH and soil nutrient concentration (i.e., Zn and Pi deficiency) influence AM-mediated Zn content in different plant tissues.

2.1.2.6 Lipid Metabolism

Glomeromycetes can be certified as 'oleogenic' fungi as approximately 25% of their dry weight consists of lipids (Bago et al. 2002; Malbreil et al. 2014). Several experiments have revealed that lipid metabolism has an unexpected and specific regulation mechanism: C is obtained from plants as hexose but mainly stored as TAG (a compact form of C storage, allowing long-distance translocation) in hyphae and more particularly in spores (Malbreil et al. 2014) and is needed when required. Various labelling experiments have disclosed that synthesis of palmitic acid (the first produced in fatty acid synthesis and precursor to longer ones) takes place in only in IRM and is used in IRM, ERM or germinating spores (Pfeffer et al. 1999; Trépanier et al. 2005). Moreover, through their study, Tisserant et al. (2012) revealed that all the genes involved in the synthesis of fatty acids are present in *R. irregularis* and the fungus did not rely on the host plant to obtain them. However,

it is not probable and might imply regulation at post-transcriptional level. Several genes related to fatty acid metabolism such as desaturase and lipase were found to be upregulated (five- and fourfold changes, respectively), out which, only 7% that belongs to lipid transport and metabolism were found to be upregulated *in planta* (Tisserant et al. 2013; Malbreil et al. 2014).

2.2 Conclusion and Future Prospects

From the above facts, it could be concluded that AM fungi are able to form effective symbiosis with host and act as an active bridge between the soil and the plant, thereby improving nutrient dynamics in the soil ecosystem. However, there is a paucity of information regarding the mechanisms that regulate the production of signal molecules under different environmental conditions. Moreover, the data regarding exchange of resources between the two symbionts, summarized in this chapter is largely based on trials with root organ cultures or with single plants that have been colonized by single AM species. Thus, in order to have a better insight about the dynamics of AM signalling as well as nutrient exchange between the symbionts, further research needs to be conducted under natural field conditions where multiple trading partners operate simultaneously. The information, thus generated could probably be used in developing new green technologies that might play an important role in sustainable agriculture.

Acknowledgements The authors are grateful to the Department of science and technology (DST—under PURSE GRANT) and Department of Biotechnology (DBT), Government of India for providing financial assistance for undertaking the research in the above context.

References

Akiyama K, Hayashi H (2006) Strigolactones: chemical signals for fungal symbionts and parasitic weeds in plant roots. Ann Bot (Lond) 97:925–931

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Antolın-Llovera M, Ried MK, Binder A, Parniske M (2012) Receptor kinase signaling pathways in plant-microbe interactions. Annu Rev Phytopathol 50:451–473

Aono T, Maldonado-Mendoza IE, Dewbre GR, Harrison MJ, Saito M (2004) Expression of alkaline phosphatase genes in arbuscular mycorrhizas. New Phytol 162:525–534

Aroca R, Ruiz-Lozano JM, Zamarreño 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

Azcón R, Barea JM (2010) Mycorrhizosphere interactions for legume improvement. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer, Vienna, pp 237–271

Bago B, Vierheilig H, Piché Y, Azcón-Aguilar C (1996) Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus Glomus intraradices grown in monoxenic culture. New Phytol 133:273–280

Bago B, Pfeffer P, Shachar-Hill Y (2000) Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol 124:949–957

Bago B, Zipfel W, Williams RM, Jun J, Arreola R, Lammers PJ, Pfeffer PE, Shachar-Hill Y (2002) Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. Plant Physiol 128:108–124

Bago B, Pfeffer PE, Abubaker J, Allen JW, Brouillette J, Douds DD, Lammers PL, Shacher-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Am Soc Plant Biol 131:1496–1507

Bakshi M, Sherameti I, Meichsner D, Thürich J, Varma A, Johri AK, Yeh K-W, Oelmüller R (2017) *Piriformospora indica* reprograms gene expression in *Arabidopsis* phosphate metabolism mutants but does not compensate for phosphate limitation. Front Microbiol 8:1262. https://doi.org/10.3389/fmicb.2017.01262

Balestrini R, Lanfranco L (2006) Fungal and plant gene expression in arbuscular mycorrhizal symbiosis. Mycorrhiza 16:509–524

Barea JM (2010) Mycorrhizas and agricultural fertility. In: González-Fontes A, Gárate A, Bonilla I (eds) Agricultural Sciences: topics in modern agriculture. Studium, Houston, TX, pp 257–274

Barea JM, Azcón R, Azcón-Aguilar C (2005) Interactions between mycorrhizal fungi and bacteria to improve plant nutrient cycling and soil structure. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Berlin, pp 195–212

Barea JM, Pozo MJ, Azcón R, Azcón-Aguilar C (2013) Microbial interactions in the rhizosphere. In: de Bruijn F (ed) Molecular microbial ecology of the rhizosphere. Wiley-Blackwell, Hoboken, NJ, pp 29–44

Barea JM, Pozo MJ, López-Ráez JA, Aroca R, Ruíz-Lozano JM, Ferrol N, Azcón R, Azcón-Aguilar C (2014) Arbuscular mycorrhizas and their significance in promoting soil-plant system sustainability against environmental stresses. In: Rodelas MB, González-López J (eds) Beneficial plant-microbial interactions ecology and applications. CRC, Taylor & Francis, Boca Raton, FL, pp 353–387

Behie SW, Bidochka MJ (2014) Nutrient transfer in plant—fungal symbioses. Trends Plant Sci 19:734–740

Behie SW, Zelisko PM, Bidochka MJ (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577

Benedetto A, Magurno F, Bonfante P, Lanfranco L (2005) Expression profiles of a phosphate transporter gene (GmosPT) from the endomycorrhizal fungus *Glomus mosseae*. Mycorrhiza 15:620–627

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. https://doi.org/10.3389/fmicb.2015.01559

Bieleski RL (1973) Phosphate pools, phosphate transport and phosphate. Annu Rev Plant Physiol 24:225–252

Bonfante P, Desirò A (2015) Arbuscular mycorrhizas: the lives of beneficial fungi and their plant host. In: Lugtenberg B (ed) Principles of plant-microbe interactions. Springer, Cham, pp 235–245

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Comm 1:48. https://doi.org/10.1038/ncomms1046

Bonfante P, Balestrini R, Mendgen K (1994) Storage and secretion processes in the spore of Gigaspora margarita Becker & Hall as revealed by high-pressure freezing and freeze substitution. New Phytol 128:93–101

Breuillin-Sessoms F, Floss DS, Gomez SK, Pumplin N, Ding Y, Levesque-Tremblay V, Noar RD, Daniels DA, Bravo A, Eaglesham JB, Benedito VA, Udvardi MK, Harrison MJ (2015) Suppression of arbuscule degeneration in Medicago truncatula phosphate transporter 4 mutants is dependent on the ammonium transporter 2 family protein AMT2;3. Plant Cell 27:352–1366

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. New Phytol 173:11–26

Bücking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy 5:587–612

Bücking H, Shachar-Hill Y (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus Glomus intraradices is stimulated by increased carbohydrate availability. New Phytol 165:899–912

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. In: Dhal NK, Sahu SC (eds) Plant science. Intech, Rijeka, pp 107–539

Cameron DD (2010) Arbuscular mycorrhizal fungi as (agro) ecosystem engineers. Plant Soil 333:1–5

Carbonnel S, Gutjahr C (2014) Control of arbuscular mycorrhiza development by nutrient signals. Front Plant Sci 5(462). https://doi.org/10.3389/fpls.2014.00462

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

Cimen I, Pirinc V, Doran I, Turgay B (2010) Effect of soil solarization and arbuscular mycorrhizal fungus (Glomus intraradices) on yield and blossom-end rot of tomato. Int J Agric Biol 12:551–555

Corrêa A, Cruz C, Ferrol N (2015) Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza 25:499–515

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

Eriksen J, Askegaard M (2000) Sulphate leaching in an organic crop rotation on sandy soil in Denmark. Agric Ecosyst Environ 78:107–114

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

Ferrol N, Barea JM, Azcón-Aguilar C (2000) The plasma membrane H+-ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*. Curr Genet 37:112–118

Frey B, Schüepp H (1993) Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with Zea mays L. New Phytol 124:221–230

Garg N, Chandel S (2010) Arbuscular mycorrhizal networks: process and functions. A review. Agron Sustain Dev 30:581–599

Gaude N, Bortfeld S, Duensing N, Lohse M, Krajinski F (2012) Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo extensive and specific reprogramming during arbuscular mycorrhizal development. Plant J 69:510–528

Genre A (2012) Signalling and the re-structuring of plant cell architecture in am symbiosis. In: Perotto S, Baluška F (eds) Signaling and communication in plant symbiosis, Signaling and communication in plants, vol 11. Springer, Berlin, pp 51–71

Genre A, Bonfante P (2010) The making of symbiotic cells in arbuscular mycorrhizal roots. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 57–71

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in Medicago truncatula root epidermal cells before infection. Plant Cell 17:3489–3499

Genre A, Chabaud M, Balzergue C, Puech-Pagès V, Novero M, Rey T, Fournier J, Rochange S, Bécard G, Bonfante P, Barker DG (2013) Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in Medicago truncatula roots and their production is enhanced by strigolactone. New Phytol 198:190–202

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

Giovannetti M, Tolosano M, Volpe V, Kopriva S, Bonfante P (2014) Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in Lotus japonicus. New Phytol 204:609–619

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

Gómez-Roldán V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194

Grønlund M, Albrechtsen M, Johansen IE, Hammer E, Nielsen TH, Jakobsen I (2013) The interplay between P uptake pathways in mycorrhizal peas: a combined physiological and gene-silencing approach. Physiol Plant 149:234–248

Guether M, Neuhauser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P (2009) A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. Plant Physiol 150:73–83

Gutjahr C (2014) Phytohormone signaling in arbuscular mycorrhiza development. Curr Opin Plant Biol 20:26–34

Gutjahr C, Parniske M (2013) Cell and developmental biology of the arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617

Gutjahr C, Casieri L, Paszkowski U (2009) *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. New Phytol 182:829–837

Hamel C (2004) Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone. Can J Soil Sci 84:383–395

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 (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, Mrosk C, Isayenkov S, Strack D (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68:101–110

Hawkins HJ, Johansen A, George E (2000) Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226:275–285

He XH, Critchley C, Bledsoe C (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). Crit Rev Plant Sci 22:531–567

Helber N, Wippel 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

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci USA 107:13754–13759

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299

Jakobsen I, Hammer EC (2015) Nutrient dynamics in arbuscular mycorrhizal networks. In: Horton TR (ed) Mycorrhizal networks, ecological studies, vol 224. Springer, Dordrecht, pp 91–131

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ (2007a) A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 104:1720–1725

Javot H, Pumplin N, Harrison M (2007b) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. Plant Cell Environ 30:310–322

Javot H, Penmetsa RV, Breuillin F, Bhattarai KK, Noar RD, Gomez SK, Zhang Q, Cook DR, Harrison MJ (2011) Medicago truncatula mtpt4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. Plant J 68:954–965

Jeffries P, Barea JM (2012) Arbuscular mycorrhiza—a key component of sustainable plant-soil ecosystems. In: Hock B (ed) The mycota. Springer, Berlin, pp 51–75

Johansen A, Jakobsen I, Jensen ES (1993) Hyphal transport by vesicular-arbuscular mycorrhizal fungus on N applied to the soil as ammonium or nitrate. Biol Fert Soils 16:66–70

Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. Trends Plant Sci 10:22–29

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882

Koegel S, Ait Lahmidi N, Arnould C, Chatagnier O, Walder F, Ineichen K, Boller T, Wipf D, Wiemken A, Courty PE (2013) The family of ammonium transporters (AMT) in Sorghum bicolor: two AMT members are induced locally, but not systemically in roots colonized by arbuscular mycorrhizal fungi. New Phytol 198:853–865

Kohlen W, Charnikhova T, Lammers M, Pollina T, Tóth P, Haider I, Pozo MJ, de Maagd RA, Ruyter-Spira C, Bouwmeester HJ, López-Ráez JA (2012) The tomato CAROTENOID CLEAVAGE DIOXYGENASE 8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. New Phytol 196:535–547

Koltai H, Kapulnik Y (2009) Effect of arbuscular mycorrhizal symbiosis on enhancement of tolerance to abiotic stresses. In: White JF, Torres MS (eds) Defensive mutualism in microbial symbiosis. CRC, Taylor & Francis, Boca Raton, FL, pp 217–234

Kosuta S, Hazledine S, Sun J, Miwa H, Morris RJ, Downie JA, Oldroyd GE (2008) Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. Proc Natl Acad Sci USA 105:9823–9828

Kranabetter JM (2014) Ectomycorrhizal fungi and the nitrogen economy of conifers—implications for genecology and climate change mitigation. Botany 92:417–423

Kumar A, Dames JF, Gupta A, Sharma S, Gilbert JA, Ahmad P (2015) Current developments in arbuscular mycorrhizal fungi research and its role in salinity stress alleviation: a biotechnological perspective. Crit Crit Rev Biotechnol 35:461–474

Lammers PJ, Jun J, Abubaker J, Arreola R, Gopalan A, Bago B, Hernandez-Sebastia C, Allen JW, Douds DD, Pfeffer PE, Shachar-Hill Y (2001) The glyoxylate cycle in an arbuscular mycorrhizal fungus. Carbon flux and gene expression. Plant Physiol 127:1287–1298

Lanfranco L, Vallino M, Bonfante P (1999) Expression of chitin synthase genes in the arbuscular mycorrhizal fungus Gigaspora margarita. New Phytol 142:347–354

Lehmann A, Rillig MC (2015) Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops—a meta-analysis. Soil Biol Biochem 81:147–158

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants—a meta-analysis. Soil Biol Biochem 69:123–131

Leigh EG (2010) The evolution of mutualism. J Evol Biol 23:2507–2528

Leigh J, Hodge A, Fitter AH (2009) Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. New Phytol 181:199–207

López-Pedrosa A, González-Guerrero M, Valderas A, Azcón-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

López-Ráez JA (2016) How drought and salinity affect arbuscular mycorrhizal symbiosis and strigolactone biosynthesis? Planta 243:1375–1385

López-Ráez JA, Pozo MJ (2013) Chemical signalling in the arbuscular mycorrhizal symbiosis: biotechnological applications. In: Aroca R (ed) Symbiotic endophytes, Soil biology, vol 37. Springer, Berlin, pp 215–232

López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 61:2589–2601

López-Ráez JA, Bouwmeester H, Pozo MJ (2012) Communication in the rhizosphere, a target for pest management. In: Lichtfouse E (ed) Agroecology and strategies for climate change, Sustainable agriculture reviews, vol 8. Springer, Dordrecht, pp 109–133

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63

Malbreil M, Tisserant E, Martin F, Roux C (2014) Genomics of arbuscular mycorrhizal fungi. Out of the shadows. Adv Bot Res 70:259–290

Marschner H, Dell B (1994) Nutrient uptake and mycorrhizal symbiosis. Plant Soil 159:89–102

Marzluf GA (1996) Regulation of nitrogen metabolism in mycelial fungi. In: Brambl R, Marzluf GA (eds) Biochemistry and molecular biology, The mycota, vol 3. Springer, Berlin, pp 357–368

Matsumura A, Taniguchi S, Yamawaki K, Hattori R, Tarui A (2013) Nitrogen uptake from amino acids in maize through arbuscular mycorrhizal symbiosis. Am J Plant Sci 4:2290–2294

McFarland JW, Ruess RW, Kielland K, Pregitzer K, Hendrick R, Allen M (2010) Cross-ecosystem comparisons of in situ plant uptake of amino acid-N and NH4. Ecosystems 13:177–193

Miransari M (2013) Arbuscular mycorrhizal fungi and uptake of nutrients. In: Aroca R (ed) Symbiotic endophytes, Soil biology, vol 37. Springer, Berlin, pp 253–270

Mohanta TK, Bae H (2015) Functional genomics and signaling events in mycorrhizal symbiosis. J Plant Interact 10(1):21–40

Nagy R, Karandashov V, Chague W, Kalinkevich K, Tamasloukht M, Xu G, Jakobsen I, Levy AA, 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

Nasim G (2013) Host allelopathy and arbuscular mycorrhizal fungi. In: Cheema ZA, Farooq M, Wahid A (eds) Allelopathy. Springer, Berlin, pp 429–450

Nygren CMR, Edqvist J, Elfstrand M, Heller G, Taylor AF (2007) Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. Mycorrhiza 17:241–248

Oldroyd GED (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263

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 Nat Acad Sci USA 99:13324–13329

Pérez-Tienda J, Testillano PS, Balestrini R, Fiorilli V, Azcón-Aguilar C, Ferrol N (2011) GintAMT2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*. Fungal Genet Biol 48:1044–1055

Pfeffer P, Douds DD, Becard G, Shachar-Hill Y (1999) Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol 120:587–598

Pozo MJ, López-Raéez JA, Azcón C, García-Garrido JM (2015) Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. New Phytol 205:1431–1436

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

Pumplin N, Mondo SJ, Topp S, Starker CG, Gantt JS, Harrison MJ (2010) Medicago truncatula Vapyrin is a novel protein required for arbuscular mycorrhizal symbiosis. Plant J 61:482–494

Purin S, Rillig MC (2007) The arbuscular mycorrhizal fungal protein glomalin: limitations, progress and a new hypothesis for its function. Pedobiologia 51:123–130

Ravnskov S, Jakobsen I (1995) Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytol 129:611–618
Ravnskov S, Wu Y, Graham JH (2003) Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots. New Phytol 157:539–545
Read D (1998) Biodiversity—plants on the web. Nature 396:22–23
Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal associations in 'lower' land plants. Philos Trans R Soc Lond B Biol Sci 355:815–831
Reddy S, Schorderet M, Feller U, Reinhardt D (2007) A petunia mutant affected in intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi. Plant J 51:739–750
Redeker D, Kodner R, Graham L (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921
Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339
Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, De Pascale S, Bonini P, Colla G (2015) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic 196:91–108
Schaarschmidt S, Roitsch T, Hause B (2006) Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots. J Exp Bot 57:4015–4023
Schüβler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444:933–936
Shachar-Hill Y, Pfeffer PE, Douds D, Osman SF, Doner LW, Ratcliffe RG (1995) Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leek. Plant Physiol 108:7–15
Shah MA (2014) Mycorrhizas: novel dimensions in the changing world. Springer, New Delhi, p 5
Siciliano V, Genre A, Balestrini R, Cappellazzo G, DeWit PJGM, Bonfante P (2007) Transcriptome analysis of arbuscular mycorrhizal roots during development of the prepenetration apparatus. Plant Physiol 144:1455–1466
Sieh D, Watanabe M, Devers EA, Brueckner F, Hoefgen R, Krajinski F (2013) The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of Medicago truncatula. New Phytol 197:606–616
Singh S, Katzer K, Lambert J, Cerri M, Parniske M (2014) CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. Cell Host Microbe 15:139–152
Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, London
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, New York, p 800
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 SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20
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
Smith SE, Jakobsen I, Gronlund 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
Solaiman MZ, Saito M (1997) Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. New Phytol 136:533–538
Song YY, Zeng RS, Xu JF, Li J, Shen X, Yihdego WG (2010) Inter plant communication of tomato plants through underground common mycorrhizal networks. PLoS One 5(10):e13324

St. John TV, Coleman DC, Reid CPP (1983) Association of vesicular–arbuscular mycorrhizal hyphae with soil organic particles. Ecology 64:957–959

Tian C, Kasiborski B, Koul R, Lammers PJ, Bücking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175–1187

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, Gollotte A (1993) In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycol Res 97:245–250

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, Symeonidid A, Balestrini R, Charron P, Duensing N, dit Frey NF, Gianinazzi-Pearsoni V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, 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, Tuskans GA, Young JPW, Sanders IR, Henrissat B, Rensing SA, Grigorievc 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 USA 110:20117–20122

Trépanier M, Bécard G, Moutoglis P, Willemot C, Gagné S, Avis TJ, Rioux JA (2005) Dependence of arbuscular-mycorrhizal fungi on their plant host for palmitic acid synthesis. Appl Environ Microbiol 71:5341–5347

Valentine AJ, Mortimer PE, Kleinert A, Kang Y, Benedito VA (2013) Carbon metabolism and costs of arbuscular mycorrhizal associations to host roots. In: Aroca R (ed) Symbiotic endophytes, Soil biology, vol 37. Springer, Berlin, pp 233–252

Vogel JT, Walter MH, Giavalisco P, Lytovchenko A, Kohlen W, Charnikhova T, Simkin AJ, Goulet C, Strack D, Bouwmeester HJ, Fernie AR, Klee HJ (2010) SlCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. Plant J 61:300–311

Volpe V, Giovannetti M, Sun X-G, Fiorilli V, Bonfante P (2016) The phosphate transporters LjPT4 and MtPT4 mediate early root responses to phosphate status in non-mycorrhizal roots. Plant Cell Environ 39:660–671

Walder F, Brulé D, Koegel S, Wiemken A, Boller T, Courty P-E (2015) Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the Pht1 family in sorghum and flax. New Phytol 205:1632–1645

Walker C (1995) AM or VAM: what's in a word? In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 25–26

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

Xie X, Huang W, Liu F, Tang N, Liu Y, Lin H, Zhao B (2013) Functional analysis of the novel mycorrhiza-specific phosphate transporter AsPT1 and PHT1 family from *Astragalus sinicus* during the arbuscular mycorrhizal symbiosis. New Phytol 198:836–852

Yang Q, Chen ZZ, Zhou XF, Yin HB, Li X, Xin XF, Hong XH, Zhu JK, Gong Z (2009) Overexpression of SOS (salt overly sensitive) genes increases salt tolerance in transgenic Arabidopsis. Mol Plant 2:22–31

Yang S-Y, Grønlund M, Jakobsen I, Suter Grotemeyer M, Rentsch D, Miyao A, Hirochika H, Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski U (2012) Non redundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the PHOSPHATE TRANSPORTER1 gene family. Plant Cell 24:4236–4251

Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y, Yoneyama K (2007) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227:125–132

Yoneyama K, Xie X, Kim H, Kisugi T, Nomura T, Sekimoto H, Yokota T, Yoneyama K (2012) How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation? Planta 235:1197–1207

Zaefarian F, Rezvani M, Rejali F, Ardakani MR, Noormohammadi G (2011) Effect of heavy metals and arbuscular mycorrhizal fungal on growth and nutrients (N, P, K, Zn, Cu and Fe) accumulation of alfalfa (*Medicago sativa* L.) Am Eurasian J Agric Environ Sci 11:346–352

Chapter 3
Capturing Plant Genetic Potential of Upland Rice for Exploiting Arbuscular Mycorrhiza Responsiveness to Improve Rice Variety for Higher Phosphorus (P) Acquisition Under P Limiting Environments

Neha Nancy Toppo and Dipankar Maiti

Abstract Efforts have been made to elaborate the benefits of arbuscular mycorrhiza (AM)—upland rice associations and underpin the importance of exploiting the genetic variability of upland rice varieties upon mycorrhization. The upland ecology is agriculturally least intervened and upland rice naturally draws benefits (especially P-acquisition) from the minimally disturbed native AM-flora, paving the way for the development of improved varieties with an inherent potential for native AM-aided enhanced P-nutrition. For this, understanding the physiological traits linked to AM-response is essential. The results indicated that upland rice varieties with high growth rate, high P-demand and low soil exploration capacity (poor root length density) are AM-responsive, though none of these physiological traits individually were significantly correlated with mycorrhiza responsiveness (% MR) suggesting their ineffectiveness as stand-alone valid screening criteria for AM responsiveness. However, the traits, may prove useful when studied in combination. Molecular characterization with 15 SSR markers revealed Sathi-34-36 and Jonga as genotypically most diverse (in terms of AM responsiveness) indicating possibilities of utilizing these two upland rice varieties for developing mapping population for QTL analysis which may serve as basic molecular tool to develop AM-responsive upland rice varieties through marker-assisted selection.

N.N. Toppo
Central Tasar Research and Training Institute (Central Silk Board), Ministry of Textiles, GOI, Ranchi 825 303, Jharkhand, India

D. Maiti (✉)
Central Rainfed Upland Rice Research Station (ICAR – National Rice Research Institute), PB 48, Hazaribag 825 301, Jharkhand, India
e-mail: dipankar_maiti@live.in

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_3

3.1 Introduction

Mycorrhiza, derived from the Greek word *mykes* and *rhiza* meaning fungus and root respectively (Frank 1885), are obligate biotrophs forming symbiotic associations between plant roots and fungi for the past 460 million years (Simon et al. 1993), indicating that plants have formed associations with arbuscular mycorrhizal fungi (AMF) since the plants first colonized land (Remy et al. 1994; Pirozynski and Malloch 1975). Mycorrhizal fungi form symbiotic relationships with plant roots in a fashion similar to that of root nodule bacteria within legumes. This group of fungi (AMF) form a living bridge between plant root and bulk soil in most ecosystems (Friberg 2001). Globally, mycorrhizae occur in 80–90% of plant species (83% of dicotyledonous and 79% of monocotyledonous plants and all gymnosperms) (Wilcox 1991). AMF associations are known for improving nutrient acquisition especially of the less mobile nutrients like 'phosphorus' (P), zinc (Zn) etc. by plants apart from enhancing plant resistance to biotic (soil-borne diseases, nematodes) and abiotic (moisture stress, heavy metal toxicity etc.) stresses (Smith and Read 2008). The available P present in the soil solution (P_i) and labile P-pool (as orthophosphate ions, $H_2PO_4^-$ and HPO_4^{2-} depending on the pH) adsorbed on soil particles which is in rapid equilibrium with solution P, are made available to the plant *via* the increased surface absorption provided by the extra-radical mycelial network of AMF, from beyond the root absorption zone and the P-depletion zone (characteristically present around root systems), which is otherwise inaccessible to plant.

Upland rice, as abundantly documented (Bagyraj et al. 2015; Maiti 2015; Maiti et al. 2011a, b, 2012; Gao et al. 2007) benefits from AMF as well as it is naturally infected by arbuscular mycorrhizae (Gangopadhyay and Das 1982). Upland rice accounts for about 13% of the world rice area (David 1991) with nearly two-thirds of global upland rice area in Asia. The upland rice area in India is about 6.0 million hectares (ha), which accounts for 13 % of the total rice area in the country (Adhya et al. 2008). It is largely distributed in Eastern India and North-Eastern Hill region. Out of the total upland area (6.0 million ha), 4.3 million ha lies in Eastern India (Assam, West Bengal, Orissa, Jharkhand, Eastern Uttar Pradesh and Chhattisgarh). Upland rice in India is grown in (a) unfavorable (*unbunded*) uplands and (b) favourable (*bunded*) uplands and is predominantly rainfed, direct seeded and grown under well-drained, aerobic (*unbunded* uplands) to semi-aerobic (*bunded* uplands) soil conditions. The soil type is generally red/lateritic alfisols with acidic (*unbunded* uplands) to near-neutral pH (*bunded* uplands). Upland rice is mostly grown by resource poor farmers as subsistence farming (Maiti et al. 2007). The recent advances for agricultural productivity are mostly inaccessible to this segment of farmers. Moreover, the upland ecology being drought prone leads to poor crop nutrient acquisition especially P. Proper P-nutrition is vital in the early vegetative growth stages of rice and is crucial for rice grain yield. It promotes early plant growth and development of a strong root system. It promotes tillering, root development, early flowering and ripening. Without adequate supply of P, plants cannot

reach its maximum yield and its deficiency symptoms appear in the lower part of the plant resulting in dark green leaves, stunting, decreased leaf number and leaf blade length. Poor P supply also reduces panicle number/plant, grain number/panicle and filled grain number/panicle (Kimani et al. 2013). The constraints (lack of capital to purchase inputs, low and unstable yields resulting from soil degradation, poor nutrient acquisition, drought, diseases, weeds etc.) lead to very poor productivity (0.90 tonnes per ha) of upland rice (Singh 2009).

Addition of P-fertilizers in large amounts for enhancing rice yield is commonly practiced all around the world (Itao et al. 1999). High application rate of chemical P-fertilizers is not a solution in this ecology with acidic soil conditions, as they rapidly form insoluble complexes in soil rendering it unavailable for plant uptake (Sahrawat et al. 2001; Holford 1997) thus making fertilizers a costly input with low efficiency and average returns (Rose et al. 2013). Inordinate P supply to the soil also poses major environmental risks of rapid depletion of the finite and non-renewable rock phosphate reserve (Edixhoven et al. 2013) and increased P movement to surface and ground waters leading to degradation of quality of aquatic ecosystems by encouraging widespread eutrophication and washing-off from fields into rivers, poisoning coastal waters and leading to acid rain (Nosengo 2003). So, a balance between sufficient P for crops and avoiding excess P in soil is important (Grant et al. 2005). Thus, harnessing mycorrhizal eco-system services utilizing AMF in the form of native AMF bio-fertilizers in synergism with biological rhizospheric processes can aid in minimizing chemical fertilizer dependence and promote sustainable agriculture in the long run.

Sound association of upland rice with AM-fungi forming mycorrhizal symbiosis has been confirmed by several workers (Maiti et al. 1995; Dhillion and Ampornpan 1992; Brown et al. 1988). Partial dependence of upland rice on AMF for P-acquisition was also demonstrated (Saha et al. 2005) especially under rice based cropping systems that harbour higher AMF population and activity in soil by providing favourable soil environment in the form of supportive root system of crops in addition to rice for extended periods (Maiti et al. 2012). Positive effects of indigenous AM-inoculation manifesting in terms of increased P-acquisition in rice (Solaiman and Hirata 1997) including upland rice (Maiti et al. 2011a, 2012) have been confirmed in the past. Native AM based inoculums consisting of the indigenous AMF consortium are ecologically better adapted, cost effective compared to commercial inoculums and more suitable for low value field crops like rice. Commercial/exotic inoculums (AMF), on the other hand, also have certain disadvantages, especially in a low value crop like rice grown under uplands, and has been extensively reviewed by Maiti (2011). The reasons include (1) abundance of indigenous population in the soil of the target agro-ecology (Maiti et al. 1995), (2) propensity of the active native AMF and other natural soil micro-flora to reject intruders/exotic fungi (Muok et al. 2009; Vierheilig et al. 2000), (3) high cost and limited availability of quality inoculum, (4) off-season winter and summer crashing-off of non-stabilized, introduced AMF population under strong ecological stress like long duration post crop fallowing, soil desiccation etc. necessitating application of inoculum for each crop cycle and (5) possible threat of unintentional

introduction of harmful microbes as contaminants (Schwartz et al. 2006). Also in intensive cropping systems, the use of fertilizers and pesticides inhibits AMF (Bethlenfalvay et al. 1996; Garciaromera and Ocampo 1988). Modern crop varieties are also less dependent than older varieties on AMF because the modern varieties have been selected under conditions of high soil fertility (Zhu et al. 2001).

Higher efficacy of comparatively less disturbed native AMF population due to minimum agricultural interventions in upland rice ecology, predominantly under subsistence farming was previously documented (Maiti et al. 2011b). Thus, it has been observed that upland rice varieties are mostly responsive to AMF. This has led to researchers believing that the problem of poor P-nutrition could be alleviated by additional means of using genotypes with higher AM-response and enhanced P-acquisition from the fixed P deposits in soil. Integration of AM-responsive varieties with identified AM-supportive crop culture components (Maiti et al. 2011a, 2012) would further enhance AM-aided P-nutrition of upland rice.

3.2 The Genetic Basis of Mycorrhizal Responsiveness

'Mycorrhiza responsiveness' is a term generally used to quantify variation in response to mycorrhiza formation among plant taxa (Mosse 1973). However, in functional term, mycorrhiza responsiveness (MR) is defined as a change in plant biomass that results from the symbiosis (Smith and Smith 2011) or as difference in growth response between mycorrhizal and non-mycorrhizal plants to inoculation (mycorrhizal fungi) under a given environment (Janos 1988). Janos (2007) redefined mycorrhiza responsiveness as the increased growth or P uptake of a plant resulting from AMF colonisation. Mycorrhiza responsiveness is highly variable among plant genotypes (Chu et al. 2013; An et al. 2010). Attempts to examine variation between AM host cultivars in their capacity to respond to mycorrhization have been mainly done in *Zea mays* (maize), *Hordeum vulgare* (barley) and *Triticum aestivum* (wheat) (Smith and Read 1997). 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). Mycorrhiza responsiveness is seen more often in plant genotypes that are less efficient in P nutrient uptake when non-mycorrhizal i.e. plant genotypes that inherently possess mechanisms for efficient P-acquisition are usually less dependent on AMF or less mycorrhiza responsive (deOliveria et al. 2009; Gao et al. 2007; Baon et al. 1993; Koide 1991a). Also, response to mycorrhiza tends to decline if P concentration in the plant (tissue P concentration) increases. Therefore, if the plant is well-equipped to meet its inherent P requirements and if soil P levels are not limiting, mycorrhiza responsiveness will be low. These factors along with the genetic makeup of the plant and the associating fungi result in variation in response. There have been a few attempts to dissect such response variation or functional compatibility of crop plants genetically 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 grown in stressful soils. The modern molecular genetic tools have widened the scope of identification of such hidden or obscure genes with less time and cost than the conventional methods.

Inter-and intra-species variation in AMF-response (for growth promotion and P-acquisition) in cultivars of onion (*A. fistulosum*) (Tawaraya et al. 2001), in other crops (Smith and Read 1997; Koide 1991b; McGonigle and Fitter 1990) including cereals like rice (*Oryza sativa* L.) (Dhillion 1992) and wheat (*Triticum aestivum*) (Hetrick et al. 1995) have been observed. Such varietal differences in response suggest that there is a genetic basis for the host plant AMF interaction. Extensive studies carried out by Hetrick et al. (1992, 1993, 1995) confirmed that mycorrhiza responsiveness is an inherited trait with the responsiveness to mycorrhizae being essentially governed by host plant genes. Siqueira and Saggin Jr. (2001) clarified that responsiveness relates to the plants internal nutrient demand in relation to growth rate under a given environment. Sustained research on the genetics of mycorrhiza formation over the last few years has also revealed that plant response to mycorrhizas may depend on the genomic backgrounds of the fungus, the plant and their interaction with environment (Franken and Requena 2001; Smith and Read 1997). Apart from wheat, studies on maize (Kaeppler et al. 2000) also suggested that there is a genetic basis for dependency on or responsiveness to AMF. Thus, several authors and researchers now believe and promote breeding for mycorrhiza responsiveness in crop plants for maximizing efficient nutrient uptake with augmented dependence on AMF (Chu et al. 2013; Sawers et al. 2008; Ryan and Graham 2002; Zhu et al. 2001).

Formal genetic studies with large number of cultivars of *T. aestivum* indicated that 'mycorrhiza responsiveness' genes might exist in different chromosomes of some cultivars (Hetrick et al. 1995). In double haploid (DH) mapping populations of barley varieties, differences in mycorrhiza responsiveness have been identified by formal genetic approaches (Langridge et al. 1995). Using 'near-isogenic lines' (NILs) similar host genotypic variation 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 DH genetic population of *Hordeum vulgare* and the presence of 'quantitative trait loci' (QTLs) for mycorrhiza responsiveness was indicated (Barker et al. 2002). Subsequently, QTL 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 three genomic regions involved in mycorrhiza responsiveness in onion to *Glomus intraradices* of which two QTLs were in *Allium roylei* on chromosome 2 and 3 for AM-responsiveness and biomass of mycorrhizal plants, and one QTL from *Allium fistulosum* for mycorrhiza responsiveness. Kaeppler et al. in 2000 identified QTLs for growth at low P. Response to mycorrhizal fungi in a 'recombinant inbred line' (RIL) population of B73 × Mo17 maize population led to identification of three QTLs, two QTLs which controlled growth at low P in the absence of

mycorrhizae based on shoot weight and one QTL which controlled mycorrhiza responsiveness.

These findings have opened possibilities of utilizing this genetic variability to select/breed high AM-responsive rice 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 mycorrhiza response in terms of plant supply of phosphorus in an otherwise phosphorus deficit soil, simply by getting the plants infected with appropriate mycorrhiza, for the maximum P use efficiency of applied chemical P-fertilizers.

3.3 Importance of Native AM Fungi in Upland Rice P-Acquisition

The severity of poor upland rice productivity especially due to deprived P-nutrition has been highlighted time and again (Maiti et al. 1995), wherein it forms insoluble complexes with Fe and Al under the acidic soil conditions (Maiti 2015; Sahrawat et al. 2001). Literature is also replete with the problems associated with P-acquisition from soil because of rapid formation of a P-depletion zone around the roots and inability of P ions to fill up this zone *via* diffusion from the bulk soil (Smith and Smith 2012; Smith et al. 2011). Researchers have long since touted AMF as a solution especially in P limited environments such as that of the rainfed uplands. Mycorrhizal stimulation of nutrient uptake is attributed to (1) uptake of nutrients by fungi from soil beyond the depletion zones that can develop around roots (2) production of degradative extracellular enzymes or organic acids by the fungi and/or (3) the ability of the fungi to translocate nutrients faster than they could diffuse through soil (rate of P diffusion is very slow 10^{-12}–10^{-15} m^2/s) (Jones and Smith 2004). Given that AMF are ubiquitous and commonly colonize upland rice, it now seems but natural to develop upland rice varieties which are highly responsive to the indigenous mycorrhizal population.

In the past years, there have been several instances, where the ecosystem services of the native AMF have been harnessed for enhancing P-acquisition and improving P-nutrition in upland rice via the following interventions (Maiti 2015).

3.3.1 Adoption of AM-Supportive Cropping Systems and Crop Rotations

Soils used for agricultural production have low diversity of AMF compared to natural systems (Verbruggen et al. 2010; Menéndez et al. 2001) and this problem is further aggravated because of mono-cropping or crop monoculture (Oehl et al. 2003; Burrows and Pfleger 2002; An et al. 1993). The reason being, the crop selects

for those species which favour that crop without any break (Johnson et al. 1992; Schenck and Kinloch 1980). Moreover, long fallow periods also have a detrimental effect resulting in reduction of AMF population (viable AMF propagules) (Karasawa and Takebe 2011) known as fallow disorder (Thompson 1987) wherein crops exhibit P and Zn deficiency (Gosling et al. 2006). The severity of the fallow disorder is directly related to the AMF inoculum density (Maiti et al. 2006; Thompson 1991). The detrimental effects of these factors (mono-cropping and fallow disorder) can be reduced by increasing cropping intensity through introduction of AM supportive crops, suitable to specific ecology, in the cropping rotation or cropping system (Maiti and Barnwal 2012; Maiti et al. 2006). Suitable upland rice based cropping systems/rotations identified were (1) intercropping system of rice + groundnut (*Arachis hypogeal*)/pigeon pea (*Cajanus cajan* L.) (Rana et al. 2002) and (2) 2 years crop rotation of maize (*Zea mays*) relay cropped with horse gram (*Dolichos biflorus*) in the first year followed by upland rice in the second year (Maiti et al. 2006). Maiti et al. (2012) validated native AMF-aided enhancement of P-acquisition in rainfed upland rice under AM-supportive crop rotations in farmers' field condition. Similar observation of higher AMF colonization and concomitant enhanced P uptake in transplanted rice (grown under rainfed, drought prone medium lands having intermittent dry spells with dry soil conditions supporting AMF activities in soil) grown from seedlings raised in plots pre-cropped with AM-susceptible fodder grasses was reported by Maiti et al. (2008).

3.3.2 Optimizing Tillage Schedule

Off-season tillage is an agronomic recommendation for management of weed and soil borne pathogens particularly under rainfed ecology. Soil tillage, on the other hand, disrupts the established mycelial network (Karasawa and Takebe 2011; Evans and Miller 1988; Anderson et al. 1987). This soil disturbance induced (SDI) deleterious effects lead to delayed colonization in subsequent crops resulting in less P-acquisition (Jasper et al. 1991). A threshold of undisturbed period in terms of maintaining gap between two consecutive tillage operations (using bullock drawn country plough, tilling up to a depth of 10–15 cm) of 13 weeks has been worked out for rainfed uplands of eastern India (Maiti et al. 2011a). To maintain this gap, two options of off-season tillage schedules have been recommended under rainfed upland rice ecosystem for sustaining optimum activities of native AMF viz, (1) summer tillage alone and (2) initial tillage after harvest (rice) + summer tillage (Maiti et al. 2011a).

3.3.3 Optimizing P Amendment

An optimum dose of inorganic P, as high soil P levels inhibit mycorrhization (Karasawa and Takebe 2011; Grant et al. 2005), supporting maximum native

AMF activities, without sacrificing crop yield has been worked out for upland rice. A P-optimum of 20 kg P_2O_5/ha under AM-supportive 2 years' crop rotation of maize (*Zea mays* L.) relay cropped by horse gram (*Dolichos biflorus* L.) in the first year followed by upland rice in the second year as compared to the recommended dose of 30 kg P_2O_5/ha was worked out (Maiti and Barnwal 2012), suggesting about 33% economy in inorganic P-fertilizer application.

3.3.4 On-Farm Produced Indigenous/Native AMF Mass-Inoculum

A production protocol of AMF inoculum using native AMF, specifically suitable to upland rice was developed by Maiti et al. (2009) and its efficacy in terms of improving P nutrition of rice has been confirmed (Maiti et al. 2011b). An improvised mass inoculum was later developed (Toppo et al. 2016) for increased rice yield at lower doses as opposed to the earlier recommendations (Maiti et al. 2009).

All the above interventions are a positive indication of the benefits incurred by upland rice from the AM-symbiosis. Thus, identifying rice varieties that are highly responsive to the native AMF community only seems logical. However, it is also evident from previous literature that many authors doubt the efficacy of AMF in improving plant P-nutrition and plant growth as the mycorrhizal uptake pathway has been shown to make highly significant contributions to P uptake in non-responsive crop plant (Gao et al. 2007; Zhu et al. 2003; Pearson and Jakobsen 1993) and accept that the association may range from parasitism to mutualism (Jones and Smith 2004; Johnson et al. 1997). The initial depression in growth observed on association with AMF is mainly due to competition by AMF for carbohydrates without any return at early stage of colonization, confusing most that the association does not necessarily guarantee benefits, in which case the fungi is apparently acting parasitically. Jones and Smith (2004) clarified that the growth response to the association, whether negative or positive, will depend on the environment in which the mycorrhizal plant is found particularly when an essential nutrient is a limiting factor. This book chapter has from the beginning tried to establish the upland ecology befitting to draw benefits from the ecosystem services provided by these mycorrhizal fungi. Other factors for either a positive or negative response depend on genotype of the plant and on the genotype of the fungi.

3.3.5 Adoption of a Dichotomous Approach of Exploiting AM-Supportive Crop Culture Components and AM-Responsiveness of Crop Plant

The approach of genetic crop manipulation is based on twin attributes of AM symbiosis—lack of host specificity of AMF and variation in AM responsiveness

among genotypes of plants (Smith and Read 1997). The native AMF flora can be exploited best provided (1) the native population remains undisturbed and (2) the crop varieties are highly responsive to the native AMF both for colonization and P-acquisition. The upland ecology and upland rice system together satisfies both the criteria. The low productive upland soils, as under rice cultivation, are least intervened agriculturally and in such situations of low input systems of cultivation the native biological potential of the soils is expected to be least disturbed. The below ground biodiversity including that of the AMF have the chances of being more effectively conserved there than under high input systems of agriculture production. There is evidence of diverse (Toppo et al. 2012; Maiti et al. 1995) and effective (Maiti et al. 2012) AMF flora in such soils. It thus seems reasonable to take advantage of the indigenous and agriculturally least disturbed AMF community of the uplands for breeding upland rice varieties with higher mycorrhiza responsiveness to indigenous AMF for improved P uptake efficiency.

On the second aspect, there is sufficient evidence and as discussed in the above headings (Sect. 3.2) that plant response to arbuscular mycorrhiza is a variable trait. This variability could be best utilized to develop rice varieties with enhanced mycorrhiza responsiveness exploiting the biological potentials of AM in nutrition management of crop plants. Significant contributions in the genetic analysis of AMF symbiosis of crop plants have come from mutational studies combined with molecular analysis of mycorrhiza defective mutant of legume hosts—garden pea, vetch, clover, *Lotus japonicus* as model plants. Results of these studies have helped in identifying 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) having functional importance in plant biology indicated the possibility of their exploitation in breeding for symbiosis response.

3.4 Breeding for Mycorrhiza Responsive Rice Varieties

At this stage, it can be safely accepted that exploitation of AMF from native source should be promoted for sound ecological management of crop production under stressful situations. 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 headings. These agronomic manipulations in combination with genetic manipulation of crop varieties for enhanced AM response are a plausible approach to harness maximum ecosystem services rendered by native AMF. The agronomic and genetic manipulations for enhanced mycorrhizal nutrient acquisition and response are mutually inclusive and in combination could exploit to the full extent the AMF biodiversity in soil.

3.4.1 Screening for Physiological Markers Linked to AM-Responsiveness

Physiologically, the extent to which mycorrhizal infection influences plant performance in terms of mycorrhiza responsiveness is a function of the extent to which infection (AMF colonization) decreases plant P deficit by increasing the supply of P (Koide 1991b). All other factors remaining constant, plant phosphorus deficit is a function of demand for and tissue supply of phosphorus, both being inherently determined by physiological, morphological and phenological traits of the plant.

'Tissue phosphorus supply' refers to the actual rate of phosphorus uptake at a given time by the plant. The lowest rate of phosphorus uptake to give the maximum growth rate at the given time for a plant is its 'phosphorus demand'. When the demand of phosphorus exceeds the supply, it results in a 'phosphorus deficit'. Every plant has a potential demand for phosphorus that is required to satisfy its optimum or the potentially maximum growth rate. Plant tissue supply is the intrinsic capacity of the plant to draw phosphorus at the given time and under given circumstances. P-demand of a plant (the quantity of P needed to satisfy the maximum potential growth rate or biomass production per unit time) remaining constant, deficit will arise if the tissue supply does not match the demand. In other words, plant's intrinsic P-demand and inherent capacity to draw P from the soil are the basic determinants of tissue P-deficit at any given time and under the given circumstances. When there is a positive phosphorus deficit, an increase in phosphorus supply (uptake), should result in an increase in plant performance. Arbuscular mycorrhiza improves plant performance by reducing phosphorus deficit through increasing the tissue supply, thus plants which are less efficient in nutrient uptake when non-mycorrhizal become more responsive to AMF inoculation (Gao et al. 2007) in order to meet its P-deficit. It is hence, obvious, that plant genotypes varying in mycorrhiza response shall be due to variations or intrinsic differences in P-demand and tissue P supply efficiency among the genotypes. So, plant physiological traits which either independently or in combination determine/control both demand and supply of P can be considered as the basic phenotypic traits which are expected to be linked to mycorrhiza responsiveness. Another major determinant of capacity of plant to draw P from soil is its root character in terms of root length density (RLD). Plants having higher RLD are expected to explore soil more and draw higher amount of P from soil (Baldwin 1975).

By extending this physiological truism to the different genotypes of a plant taxon (in this case- rice) and looking at their inherent traits for phosphorus deficit in relation to mycorrhiza responsiveness, we can approach to understand the genetic basis of mycorrhiza responsiveness in rice at first order level. In this regard, we wanted to test the following hypothesis that 'genotypes with high growth rate having high P-demand and low soil exploration capacity must be AM-responsive'. In this light, experiments were carried out with 20 rice varieties belonging to five categories (Table 3.1) representing diverse genetic base, which were screened and studied for three physiological traits (growth rate at vegetative phase, P-demand

Table 3.1 List of germplasm used for identifying physiological traits linked to AM-responsiveness

Category	Germplasm/variety
Category A: Indigenous and exotic land races	Brown Gora, Jonga, Haskalma, Kalakeri, Saita, Atte, Thara, Azucena
Category B: Selection from land races	Sathi 34-36
Category C: Improved tall inbred varieties (pre-green revolution era)	Dular, Sattari, Mahsuri
Category D: Modern high yielding semi-dwarf varieties	T(N)1, IR 36, IR 20, CR143-2-2. Swarna
Category E: Modern high yielding semi-tall varieties	Sadabahar, Kalinga III, Jonga

and root length density) to validate the hypothesis and identify physiological traits linked to AM responsiveness in rice with an objective to work out possible screening criteria for AM-responsive upland rice genotypes. The 20 initially selected rice varieties were analysed for growth rate. Six working varieties which confirmed their growth rate gradations on repetition of experiment were finally selected for analysis of root length density, P-demand and AM-responsiveness.

3.4.1.1 Phenotypic Analysis of Physiological Traits Hypothesised to be Linked to %MR of Selected Working Genotypes

Trait Assessment

(i) ***Growth rate (GR)***: The 20 selected rice varieties were grown in black plastic tubes (42 × 12 cm) each containing two kg soil. Soil moisture was maintained at 25% of water holding capacity (WHC) by weighing method. Five replications of each variety were maintained. N:P_2O_5:K_2O fertilizer dosage at 60:30:15 kg/ha was applied to the tubes in the forms of Di-ammonium phosphate (N & P_2O_5), urea (N) and Muriate of potash (K_2O). The plants were maintained in glasshouse conditions (28–35°C). Aerial Dry matter production at 16 and 34 days after germination (DAG) was worked out and GR (dry matter accumulation in g/day/plant) computed. The experiment was repeated to confirm gradation/classification of the varieties in 11 replications each. Six genotypes were selected based on GR (aerial dry matter production) and classified as (1) fast (Sathi 34-36, Mahsuri), (2) medium (Jonga, Vandana) and (3) slow (Thara and T(N)1) (Table 3.2) based on least significant differences in values calculated using ANOVA (Cropstat 7.2). The results of repeated experiment confirmed the GR gradation of the varieties.

(ii) ***Root length density (RLD)***: The six working varieties were analysed for RLD by adopting the same methodology as used for growth rate analysis with five replications each. Whole roots were harvested at 34 DAG. The total root length (primary, secondary and tertiary) was measured using graph sheets

Table 3.2 Growth rate grade of selected rice varieties

S.no.	Rice varieties	Dry matter production rate (DMPR) (g/plant/day)				Remarks
		Repeat expt.		Preliminary expt.		
		DMPR	Grade	DMPR	Grade	
1	Sathi 34-36	0.033e	Fast	0.067d	Fast	Confirmed comparative grade
2	T(N)1	0.022a	Slow	0.024a	Slow	Confirmed comparative grade
3	Jonga	0.026bc	Medium	0.045bc	Medium	Confirmed comparative grade
4	Vandana	0.029cd	Medium	0.042abc	Medium	Confirmed comparative grade
5	Thara	0.024ab	Slow	0.029ab	Slow	Confirmed comparative grade
6	Mahsuri	0.031de	Medium-Fast	0.049cd	Medium-Fast	Confirmed comparative grade
LSD 5%		0.0033		0.0191		
SE		0.00115		0.00682		

with 1 mm^2 sub-divisions. Root length per unit volume of soil was calculated to work out the RLD. The experiment was repeated with five replications each for confirmation. Total root length, for the repeated experiment was worked out with the help of 'root scanner' (Model: EPSON PERFECTION V700) using image analyzing software—'WinRHIZO Reg V.2009C' (Regent Instrument, Canada Inc.) and RLD was computed. Based on results of repeated experiment and its comparison with preliminary experiment, varieties were grouped into high, medium and low RLD based on the least significant differences in the values calculated using ANOVA (Cropstat7.2). Jonga confirmed to have high RLD (Table 3.3). The other three genotypes, Vandana (medium RLD), T(N)1 and Mahsuri (low RLD) confirmed comparative gradations. Sathi 34-36 showed slight variation ranging from high to medium RLD while Thara showed marked inconsistency in its values (Table 3.3).

(iii) ***P-Demand***: Standard curve for stable available P of test soil with low P content (3.89 ppm) was worked out by adding external P (Potassium di-hydrogen orthophosphate) at 10, 20, 40, 80 and 160 ppm. The Mehlich 1'P' content of soils (double acid extractable P using 0.05 N HCl and 0.025 N H_2SO_4) was estimated colorimetrically using the blue colour ascorbic acid method. The experiment using the selected six working varieties was conducted with 11 replications per variety at graded P levels adjusted on the basis of the P equilibrium curve. P-demand optimum in terms of P turnover (mg/day/plant) to support potential maximum shoot growth was worked out after 40 days, by plotting per day increase in shoot growth (dW/dt; mg/day/plant) against per day increase in shoot P content (dP/dt; mg P/day/plant).

Table 3.3 Root length density of selected six rice varieties

S. no.	Rice variety	Root length density (RLD) (mm/cc soil) at 25 DAG				Remarks
		Repeated expt. (Yr. 2)		Preliminary expt. (Yr. 1)		
		RLD	Grade	RLD	Grade	
1	Jonga	3.555e	High	5.867c	High	Confirmed comparative grade
2	Sathi 34-36	2.181cd	Medium	5.455c	High	–
3	Thara	1.060a	Low	5.423c	High	–
4	Vandana	2.171cd	Medium	4.625b	Medium	Confirmed comparative grade
5	T(N)1	1.808c	Low	3.886ab	Low	Confirmed comparative grade
6	Mahsuri	1.332ab	Low	3.493a	Low	Confirmed comparative grade
	5% LSD	0.556		0.584		
	Pr > F	0.000		0.000		

Shoot P content was calculated by following the yellow colour Vanado-molybdo-phosphoric method (Jackson 1971). The experiment was repeated with the higher P demand variety Sathi 34-36 using the same standard curve for stable available P of test soil at higher P concentrations adjusted to 80, 160, 240 and 320 ppm to confirm P demand optimum.

Results showed fast growing cultivar (Sathi 34-36) with higher rate of shoot P-turnover to support their potential maximum shoot growth rate having P-demand optima >0.175 mg/day/plant (Fig. 3.1). The medium to slow growing genotypes (Vandana, Jonga and Mashuri) showed lower rate of shoot P-turnover to support their potential maximum shoot growth rate having P-demand optima <0.150 mg/day/plant (Fig. 3.1). The experiment on P demand optimum determination of Sathi 34-36, the higher P demand variety, was repeated at higher P concentrations adjusted to higher soil P concentration of 80, 160, 240 and 320 ppm to confirm P demand optimum. Sathi 34-36 consistently showed higher rate of shoot P-turnover (dP/dt) to support their potential maximum shoot growth rate (dW/dt) having P-demand optima of 0.175 mg/day/plant under both lower (10–160 ppm) (Fig. 3.1) and higher (80–320 ppm) (Fig. 3.2) test ranges of soil P concentrations as compared to that of lower P demand varieties Vandana (<0.1) and Jonga (0.125) (not shown in figure).

(iv) ***AM-responsiveness***: Estimation of AM-responsiveness of the working genotypes was done on the basis of total dry matter accumulation in terms of %MR (mycorrhizal responsiveness) using the following formula (Hetrick et al. 1992):

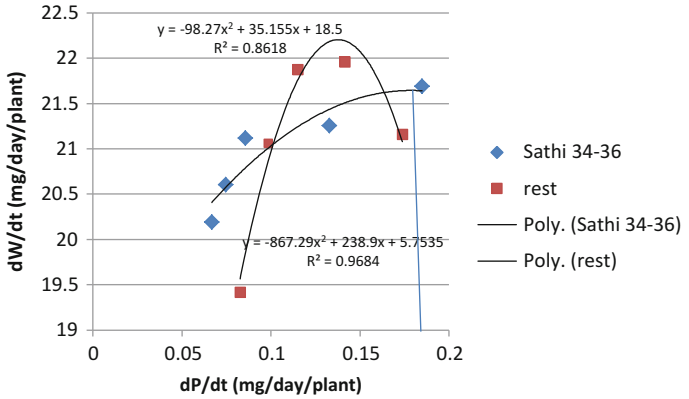


Fig. 3.1 Comparison of P-demand optima (dP/dt) of high P-demand variety (Sathi 34-36) with that of average values of lower P-demand varieties (Vandana, Jonga, Thara, T(N)1 and Mahsuri) under test P concentration range of 10–160 ppm

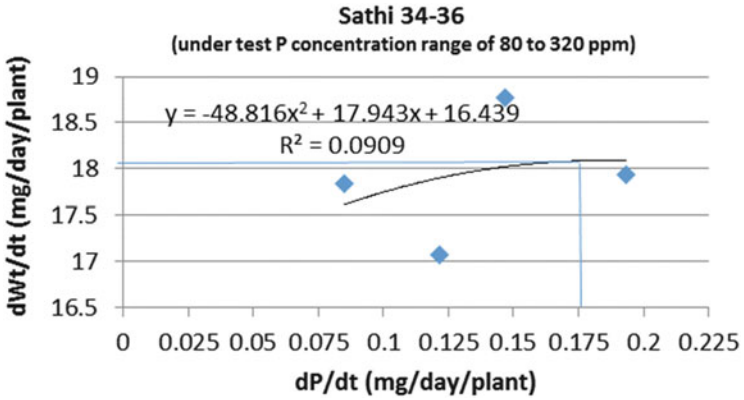


Fig. 3.2 P-demand optima of the high P demand variety (Sathi 34-36) based on means of three plants dWt/dt by dP/dt at higher range (80–400 ppm) of test concentration

$$\mathrm{MR}(\%) = \frac{(Treatment - control)}{Control} \times 100,$$

where, treatment is inoculation with AM fungi (AM+) and control is no inoculation (AM–).

The inoculum contained spores of *Glomus intraradices* (29.5 spores/g inoculum and inoculum potential (MPN) of 487.6 infective propagule number/g inoculum) procured from The Energy and Resources Institute (TERI), New Delhi, India. Seven g of inoculum was added to each tube containing two kg soil. The

same soil with low soil P content and low inoculum potential (1.76 infective propagule number/g soil) used in the P demand experiment was used in this experiment. The experiment was carried out following the same procedure used in growth rate study in black plastic tubes under glasshouse conditions with five replications per variety. Whole plants were harvested at 34 DAG and total dry weight computed to find out the %MR. The experiment was repeated for confirmation at the end of which 'Sathi-34-36' (variety developed through pure line selection from local landrace) consistently (in both experiments) showed significantly positive response to AM inoculation (based on MR%). Out of the remaining five varieties, two varieties *viz*.; Jonga, T(N)1 confirmed their non-responsiveness. The other two high yielding, improved varieties (Vandana and Mashuri) showed moderate response (Table 3.4). Thara however, did not confirm the AM-response grade in repeated experiment. Based on confirmation, 'Sathi-34-36' has been considered as strongly AM-responsive and Jonga as extremely non-responsive.

(v) ***Correlation between GR, RLD, P-demand and AM-responsiveness***: Phenotypic correlations (Pearson's Correlation) between individual phenotypic traits (GR, RLD, P demand) and AM-responsiveness (%MR) were calculated based on the mean values of the genotypes across replicates using the software SAS 9.2 (SAS Institute 2010. SAS/STAT version 9.2., SAS Institute, Cary, North Carolina, USA). Correlation at 5 % significance level between individual phenotypic traits (GR, RLD, P demand) and AM-responsiveness (%MR) revealed that % MR is highly correlated with GR and P demand but poorly or negatively correlated with RLD in both the experiments (Table 3.5).

Based on the results, the hypothesis was accepted as the rice genotypes tested held true to it. It can thus be inferred that genotypes with an inherent faster GR and higher P-demand with lower RLD (strongly AM-responsive Sathi 34-36, moderately AM-responsive Mashuri) were AM-responsive on inoculation (AMF) and genotypes with slower GR and lower P-demand with higher RLD (Jonga) were AM-non-responsive. Plants with slow growth rates generally have inherently low nutrient demands (Smith et al. 2003; Chaplin 1980) due to existing efficient nutrient allocation mechanisms, hence, these plants show little mycorrhiza responsiveness. Thus, as indicated by the results, Mahsuri with its moderately faster growth rate, high P-demand (0.176 mg/day/plant) and low RLD was moderately AM-responsive, Jonga with its moderate growth rate and low P-demand (0.127 mg/day/plant) with high RLD was AM-non-responsive suggesting that the former was dependent on mycorrhizae to make up for its P-deficit due to its poor rooting system and the latter with its extensive root system leading to better soil exploration was capable of meeting its P requirement without the intervention of AM. Plants especially cereals like wheat (Hetrick et al. 1992, 1993) with high root length and density, often have no mycorrhiza responsiveness in low P soils. Hetrick et al. (1996) in their studies revealed that responsive cultivars resulted in greater biomass than non-responsive cultivars when mycorrhizal. A larger root area

Table 3.4 Confirmation of AM response grade of selected varieties based on total dry matter production

AM response		MR %					
		Selected/inbred high yielding varieties				Land races	
		Sathi 34-36	T(N)1	Vandana	Mahsuri	Thara	Jonga
Repeat experiment (34DAG)	–AM	0.509	0.437	0.513	0.450	0.481	0.520
	+AM	0.750	0.423	0.535	0.489	0.522	0.474
	Pr > F (t test)	0.021 (2.86)	0.902 (NS)	0.684 (NS)	0.450 (NS)	0.574 (NS)	0.490 (NS)
	MR %	47.50	–8.83	4.21	8.84	8.57	–8.88
Preliminary experiment (23DAG)	–AM	0.172	0.088	0.160	0.149	0.243	0.233
	+AM	0.229	0.143	0.181	0.154	0.240	0.176
	Pr > F (t test)	0.034 (2.56)	0.41 (NS)	0.125 (NS)	0.686 (NS)	0.906 (NS)	0.058 (NS)
	MR %	33.08	–23.98	13.2	3.22	–1.24	–24.47
Remarks		Confirmed	Confirmed	Confirmed	Confirmed	–	Confirmed

Data are mean of 5 replications
DAG days after germination

Table 3.5 Correlation between the four traits for both the preliminary (P) and repeat (R) experiments

		GR	RLD	%MR	P demand opt.
GR	P	1			
	R				
RLD	P	0.26 (0.63)	1		
	R	0.05 (0.93)			
%MR	P	0.72 (0.11)	0.09 (0.86)	1	
	R	0.75 (0.08)	–0.19 (0.72)		
P demand opt.	P	0.80 (0.05)	0.23 (0.66)	0.61 (0.2)	1
	R	0.80 (0.05)	–0.04 (0.94)	0.67 (0.14)	

Values in parentheses indicates the prob > r

with highly branched root systems is important for greater soil access and therefore P uptake and in turn stable yields (Lynch 2007; Ramaekers et al. 2010).

Mahsuri showed a low positive %MR implying that the net benefit in terms of biomass due to the symbiosis was less but its GR and P-demand was high (0.176 mg/day/plant) which cannot not be sufficed with its low RLD indicating that the mycorrhizal association did have a role to play in P supply even though the benefit was not apparent (Mengel and Kirby 2001). Smith and Smith (2011), in their review, have elaborately discussed about the hidden benefits of AM association in plant species with poor mycorrhizal responsiveness leading to no net benefit in terms of dry matter production. Sathi 34-36 partly conformed to the hypothesis as it had faster growth rate, higher P-demand and was AM- responsive (highly) but its RLD ranged from high to medium suggesting that in spite of being a fast growing variety with extremely high P-demand and a fairly good soil exploration capacity it was still dependent on mycorrhizae for its P supply implying that its root system alone could not maintain its optimal P-demand.

Our studies indicated that AM-responsiveness (%MR) was positively correlated with growth rate and P-demand but negatively correlated with RLD in both the experiments, but none of the parameters were significantly correlated indicating a limitation of considering individual parameters as screening criteria for AM-responsiveness. However, GR, P-demand and RLD together may be useful physiological parameters for screening of AM-responsive breeding lines for developing rice varieties with AMF-aided enhanced P-nutrition. This also indicated that mycorrhizal responsiveness being influenced by more than one plant trait is most likely governed by multiple genes or polygenes. A highly positive and almost significant correlation ($p = 0.05$) was seen between growth rate and P-demand suggesting that a plant will have a higher P-demand if its growth rate is high. This view has already been supported by Burleigh et al. (2002) and Koide (1991b). Also, a plant with high growth rate to meet its higher P-demand so as to grow optimally may most likely be dependent on the mycorrhizal symbiosis, wherein AM fungi are known to deliver P better in low available P soils and if the inherent soil exploring capacity through root is insufficient (Bolan et al. 1983). The results indicated the

feasibility of the hypothesis and thus could be accepted. It is also important to point out here that AM-responsiveness was not expressed in terms of percent root length colonization as its inappropriateness as a responsiveness indicator has been widely demonstrated (Leiser et al. 2016; Turrini et al. 2016; Smith and Smith 2011; Hetrick et al. 1996). Studies on effect of AM inoculation on root morphological characters like root diameter, root length, root volume etc. and branching pattern of upland rice varieties (Sathi 34-36, Vandana, Jonga) was also performed (Toppo et al. 2013) and revealed that Jonga in spite of being AM non-responsive benefited in terms of root morphology from AM-inoculation with a significant increase in root diameter and root surface area suggesting that mycorrhiza does alter the root system but might not always elicit a positive response in terms of increased dry matter production because of its (Jonga) inherent capability (high RLD) to meet its lower P demand for attaining optimum growth rate. In the same study, none of the root morphological parameters and branching parameters was significantly correlated indicating their limitation as suitable screening criteria for AM responsive varieties. It was also apparent that response of a plant in terms of root parameters to AM inoculation differs with varieties.

3.4.2 Screening of Molecular Markers Linked to AM-Responsiveness in Rice

Previous findings of our research (Sect. 3.4.1) revealed mycorrhiza responsiveness to be a polygenic trait in rice thus paving the way for Quantitative-trait-loci (QTL) analysis and molecular breeding *via* marker-assisted selection for improved AM-responsiveness. The first step in QTL analysis is studying the genotypic diversity between two selected phenotypes of contrasting nature i.e., Sathi 34-36, a highly AM responsive variety and Jonga, an extremely non-responsive phenotype. Once the molecular markers polymorphic between the selected parental varieties is identified, these same markers can be used to probe the progenies of the parental cross or a suitable mapping population, to develop linkage maps based on the recombination frequencies of these markers. These linkage maps can help identify markers most closely linked to the QTLs of interest. The strength of correlation between these molecular markers and the desired trait will provide a framework of markers suitable for marker assisted selection, thus the closest markers can be used for MAS.

3.4.2.1 Genotypic Diversity Analysis of Selected Working Genotypes

Though the parents are phenotypically contrasting, QTL analysis requires that parents be genotypically diverse. In this regard, from the database of rice genomics, after thorough study, 15 SSR markers (Table 3.6) were selected to assess the genetic

Table 3.6 List of 15 SSR primers used for diversity analysis

Marker	Position	Co localized/neighboring QTL
RM212	Chromosome 1	Root depth, root-shoot ratio, plant height, grain yield etc.
RM259	Chromosome 1	Grain yield parameters
RM5	Chromosome 1	Root dry weight, grain yield etc.
RM237	Chromosome 1	Rhizome/root length, total biomass yield etc.
RM312	Chromosome 1	Root-shoot ratio, panicle number, leaf senescence etc.
RM495	Chromosome 1	Root depth, root-shoot ratio, grain yield etc.
RM55	Chromosome 3	Root dry wt., biomass yield
RM178	Chromosome 5	Root volume, seed weight etc.
RM161	Chromosome 5	Rhizome angle, plant height etc.
RM454	Chromosome 6	Root dry wt., root branching etc.
RM125	Chromosome 7	Root number, Rhizome number etc.
RM447	Chromosome 8	Root thickness, tiller number etc.
RM433	Chromosome 8	Root thickness, tiller number etc.
RM215	Chromosome 9	Root dry weight, root length, seed weight etc.
RM171	Chromosome 10	Root activity, leaf area, seed weight etc.

diversity among the 20 genotypes (including six working varieties used for earlier studies in Sect. 3.4.1). The markers had the co-localized or neighbouring characters with the total biomass production, root growth parameters, and other plant growth parameters.

All the genotypes were scored for presence (1) or absence (0) of the SSR bands and this data matrix was used to construct a dendrogram based on the confirmed reproducibility of the identified polymorphic alleles using Ward's method (squared euclidean distance) with the help of STATISTICA. Genomic DNA was extracted from the leaves of the parents using Plant Mini Kit (Qiagen, Valencia, CA, USA) from 100 mg of leaf tissue according to manufacturer's instruction. The quality and quantity of DNA were estimated spectrophotometrically using NanoDrop (NanoDrop 2000, ThermoFisher Scietific, USA). The PCR reaction was performed in a 10 µl reaction mixture containing 1 µl of template DNA (50 ng), 1 µl of 10× PCR buffer, 0.6 µl of 25 mM $MgCl_2$, 0.2 µl of 10 mM dNTPs, 0.5 µl of 10 µM forward and reverse primers and 0.2 µl of Taq DNA polymerase. The volume was raised to 10 µl with autoclaved MilliQ water. The PCR amplification reaction included an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 s and 1 min extension at 72°C. Final extension was allowed at 72°C for 10 min. After amplification, the PCR products were verified for polymorphism and product size by electrophoresis in 3% (W/V) agarose gel prepared in 1× TBE buffer and stained in ethidium bromide. Bands were visualized on Gel Documentation System. All PCR reactions for each sample were repeated at least once to confirm the results. This part of study (Sect. 3.4.2.1) was conducted at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal (India) (by Late Prof. Anirudha Das) as cooperating centre of DBT funded project which was operative at CRURRS, Hazaribag (Jharkhnad, India) as lead centre.

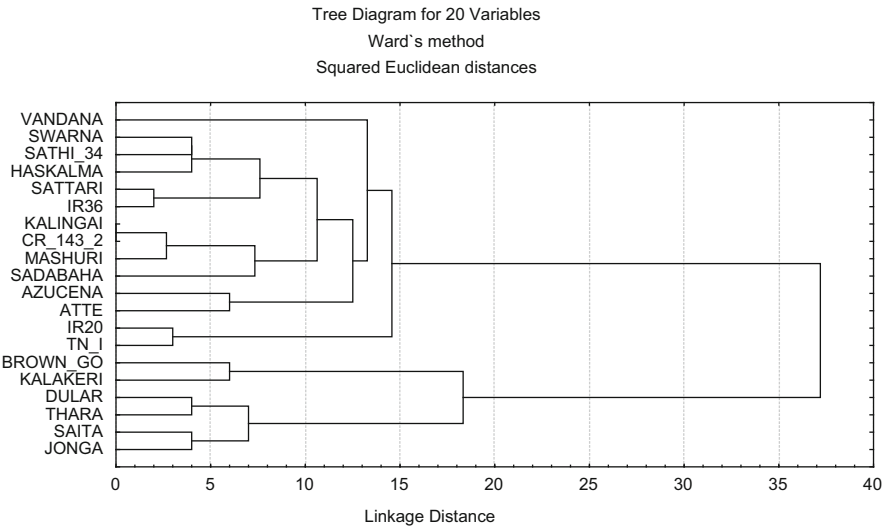


Fig. 3.3 Linkage grouping of 20 initially selected rice varieties based on DNA polymorphism study

Genomic DNA polymorphism (both single and multiple allele) using 15 selected SSR markers co-localized with root and shoot characters revealed that the six working genotypes could be easily identified with at least one of the 15 SSR markers. The dendrogram (Fig. 3.3) generated based on DNA polymorphism survey of 20 genotypes, distinguished the varieties into two distinct clusters designated as 'A' and 'B' in the figure. Though the non-responsive varieties form part of both the clusters, Sathi 34-36, the most responsive variety and Jonga the most non-responsive variety among the working varieties, based on %MR values, belonged to different clusters and were the most extreme genotypes. Thus, Sathi 34-36 and Jonga were the most phenotypically and genotypically diverse varieties which were selected as parents for further crossing and breeding programs for marker study. However, 15 markers are not enough for QTL analysis and linkage mapping, and several markers spanning the entire genome of the rice variety is required. Thus, parental polymorphism with more candidate markers was performed in the subsequent part of the study.

3.4.2.2 Parental Polymorphism Using Molecular Markers

After selection of two significantly different and contrasting phenotypes (in terms of AM-responsiveness) i.e. Sathi 34-36 and Jonga, parental polymorphism was performed i.e. a survey of polymorphism between parents selected for further crossing, was performed using molecular markers of choice. Parental polymorphism is the first step in QTL analysis and a pre-requisite for marker assisted selection;

unless the parents are polymorphic further selection of plants carrying the traits of interest is not possible in the progenies (Kumar et al. 2013). The markers of choice can be any of the DNA based genetic markers, however, it is the breeder who must decide the appropriate marker best suited to his breeding program and conditions. In rice, in recent years, microsatellite markers and single nucleotide polymorphism (SNPs) have found extensive applications in linkage map constructions and QTL/gene mapping. SSR or simple sequence repeats are microsatellite markers which are essentially tandem repeats of mono-, di-, tri-, tetra- or penta-nucleotide units dispersed throughout the genome of rice, with one SSR every 157 kb in rice, representing 2240 unique marker loci experimentally validated for rice which is publicly available (McCouch et al. 2002). In rice, a huge number of SSRs are also publicly available in Gramene database making it the easiest of markers to work with. SSRs are highly reliable (i.e., reproducible), co-dominant in inheritance, highly polymorphic (compared to other markers), generally transferable between mapping populations (Collard et al. 2008) and can be rapidly assayed by simple genotyping methods (Kong et al. 2000) making them very popular as markers of choice. Thus, SSR markers can be useful in molecular mapping and marker-assisted selection (MAS) as suggested by Aliyu et al. (2011). In the last few years a lot of work has been done with these markers for QTL analysis in rice for various traits (Sandhu et al. 2013; Mararthi et al. 2012; Kebriyaee 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. In our genotypic studies, we used 370 SSR and 91 HvSSR markers to study the parental polymorphism (unpublished). Out of these markers screened, 85 SSR and 43 HvSSR markers were found to be polymorphic between them corresponding to only 28% of the polymorphism indicating a narrow genetic variation between the two parents which could be because both parents were *indica* ecotypes. Hence, a larger number of polymorphic markers were needed to be screened to facilitate QTL analysis more precisely. Validation of more number of SSR markers can provide better genome coverage to identify other QTLs that might contribute to AM-responsiveness. For efficient utilization of SSRs it is suggested that SSR markers from the expressed portion of the genome be selected as in EST derived SSRs to facilitate their transferability across genera in comparison to SSRs developed from non-coding regions (Langridge and Chalmers 2004).

SNPs in recent years have been used in genotyping and the development of high density genetic linkage maps for mapping QTLs of various traits in rice (Li et al. 2015; Hu et al. 2013; Duan et al. 2013; Ye et al. 2011) and may also find usage in near future for identifying genetic variation for mycorrhization in rice varieties and in turn QTLs related to AM-responsiveness. An SNP is a single nucleotide base difference between two DNA sequences or individuals. The average density of SNPs in rice is 12 per 1 Mb and can provide information on polymorphism between *indica* and *japonica* subspecies as well as within *indica* and *japonica* groups (Yu et al. 2014). Though SNPs are more abundant, stable, efficient, reliable, having higher resolution, amenable to automation and are becoming increasingly

cost-effective because of the rapidly accumulating genomic re-sequencing data and several existing SNP detection technologies, they still come with their own share of economic and technical obstacles posing serious challenges to public sector or government funded breeding and research programmes (McCouch et al. 2010).

3.5 QTL Analysis and Marker-Assisted Selection

A Quantitative trait locus (QTL) is a chromosomal region where several independent loci affect phenotypic values of a quantitatively inherited trait such as grain yield and plant height (Sinha 2011). QTL analysis essentially involves linking of phenotypic (trait measurements) and genotypic (using molecular makers) data to explain the underlying genetic factors involved in the expression of complex traits (Miles and Wayne 2008). After parental polymorphism, the next step is identifying marker/trait association which is based on constructing, phenotyping and genotyping special populations called mapping populations. Langridge and Chalmer (2004) have intricately described how to do the same in their book chapter. Several types of mapping populations such as F_2 and F_3 progeny, backcross, double haploid lines (DHLs), near isogenic lines (NILs) and recombinant inbred lines (RILs) have been used for molecular genetic mappings (Knapp 1991). However, F_2, F_2 derived F_3 and F_3 population have limited use as they cannot be easily generated and maintained where as RILs and NILs can be replicated indefinitely. Molecular markers are used to partition the QTL mapping population ($<$200) into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the trait being measured (Young 1996; Tanksley 1993). Presence of significant differences between phenotypic means of the groups, depending on the marker system and type of population, indicates that the marker locus being used to partition or differentiate the mapping population is linked to a QTL controlling the trait. The closer the marker is to a QTL, the greater the chances of it being inherited along with the QTL in the progeny due to decreased probability of recombinations, with the mean of the group with the tightly linked marker being significantly different to the mean of the group with the marker. The means of the genotypic groups will not be significantly different if the marker is loosely linked to the QTL as the two segregate independently in the progeny (Boopathi 2012).

Once the markers linked to QTLs are identified, fine mapping of a QTL (using a mapping population $>$400) is required to delimit putative genes affecting the trait of interest based on statistical associations between the marker variants and the trait of interest so that the marker is within 1c M of the gene. Having identified markers physically located beside or within the genes of interest, MAS can be performed to select identifiable marker variants (alleles) in order to select for non-identifiable favourable variants of the genes of interest. Thus MAS is selection of a trait based on genotype using associated molecular markers instead of phenotype of the trait (Foolad and Sharma 2005). MAS using co-dominance markers (e.g. SSR and SNP)

can allow effective selection of recessive alleles of desired traits in the heterozygous status. No selfing or test crossing is needed to detect the traits controlled by recessive alleles, thus saving time and accelerating breeding progress (Wijerathna 2015).

Bulk segregant analysis (BSA) can also be performed as an alternative to the rigorous genotyping of each member of the large mapping population as required in QTL analysis (Michelmore et al. 1991). It is a shortcut to rapidly identify markers linked to QTLs of interest by taking two bulks each of pooled DNA of some plants from the segregating population with the same extreme phenotype, for example, one bulk with pooled DNA from highly AM-responsive phenotypes and another bulk from highly non-AM-responsive phenotypes. For BSA, as with QTL mapping, parental lines with opposing phenotypes are crossed and F2, double haploid or RILs are generated for studying trait segregation. Toppo et al. (unpublished) identified a putative molecular marker (RM 437) from the F_2 segregating population of Sathi 34-36 and Jonga that might be linked to the AM-responsiveness trait and its validation is currently in progress.

3.6 Conclusion

A better understanding of the mycorrhizal traits is thus mandatory for the phenotypic analysis of the genotypes/varieties and further development of new germplasm. Adequate phenotyping along with genotyping is essential for MAS and hence, both processes need to be thorough in selection criteria and linked marker respectively. With the completion of the International Rice Genome Project (2005), marker development and gene discovery has been immensely facilitated. MAS has found several applications in rice crop improvement but remains unutilized in capturing the genetic potential of upland rice for enhanced P acquisition. With the developing technologies, reducing cost and high throughput automated SNP genotyping, wide adoption of MAS along with conventional breeding is expected to be fruitful for the upland farmers in terms of developing highly AM-responsive rice varieties. Combined application of AM-supportive crop culture components with improved AM-responsive variety would further enhance accruing benefits from this symbiotic relationship.

References

Adhya TK, Singh ON, Swain P, Gosh A (2008) Rice in Eastern India: causes for low productivity and available options, Invited paper. J Rice Res 2:1–5

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, Singapore

An ZQ, Hendrix JW, Hershman DE, Ferriss RS, Henson GT (1993) The influence of crop-rotation and soil fumigation on a mycorrhizal fungal community associated with soybean. Mycorrhiza 3:171–182

An GH, Kobayashi S, Enoki H, Sonobe K, Muraki M, Karasawa T, Ezawa T (2010) How does arbuscular mycorrhizal colonization vary with host plant genotype? An example based on maize (*Zea mays*) germplasms. Plant Soil 327:441–453

Anderson EL, Millner PD, Kunishi HM (1987) Maize root length, density and mycorrhizal infection as influenced by tillage and soil phosphorus. J Plant Nutr 10:1349–1356

Bagyraj DJ, Sharma MP, Maiti D (2015) Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. Curr Sci 108:1288–1293

Baldwin JP (1975) A quantitative analysis of the factors affecting plant nutrient uptake from some soils. J Soil Sci 26:195–206

Baon JP, Smith SE, Alston AM (1993) Mycorrhizal responses of barley cultivars differing in P efficiency. Plant Soil 157:97–105

Barker SJ, Duplessis S, Tagu D (2002) The application of genetic approaches for investigations of mycorrhizal symbiosis. Plant Soil 244:85–95

Bethlenfalvay GH, Mihara KL, Schreiner RP, McDaniel H (1996) Mycorrhiza, biocides and biocontrol, 1. Herbicide-mycorrhiza interactions in soybean and cocklebur treated with bentazon. Appl Soil Ecol 3:197–204

Bolan N, Robson A, Barrow N (1983) Plant and soil factors including mycorrhizal infection causing sigmoidal response of plants to applied phosphorus. Plant Soil 73:187–201

Boopathi NM (2012) Genetic mapping and marker-assisted selection: basics, practice and benefits. Springer, New Delhi, p 117. https://doi.org/10.1007/978-81-322-0958-4-6

Brown MB, Quimio TH, Castro AM (1988) Vesicular arbuscular mycorrhizas associated with upland rice (*Oryza sativa* L.) Philipp Agric 77:317–332

Burleigh SH, Bechmann IE (2002) Plant nutrient transporter regulation in arbuscular mycorrhizas. Plant Soil 244:247–251

Burleigh SH, Cavagnaro T, Jakobsen I (2002) Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J Exp Bot 53:1993–1601

Burrows RL, Pfleger FL (2002) Arbuscular mycorrhizal fungi respond to increasing plant diversity. Can J Bot 80:120–130

Chaplin FS (1980) The mineral nutrition of wild plants. Annu Rev Ecol Syst 11:233–260

Chu Q, Wang X, Yang Y, Chu F, Zhang F, Feng G (2013) Mycorrhizal responsiveness of maize (*Zea mays* L.) genotypes as related to releasing date and available P content in soil. Mycorrhiza. 23:497–505

Collard BCY, Cruz CMV, McNally KL, Virk PS, Machill DJ (2008) Rice molecular breeding laboratories in the Genomics era: current status and future considerations. Int J Plant Genomics 2008:1–25. https://doi.org/10.1155/2008/524847

David CC (1991) The world rice economy: challenges ahead. In: Khush GS, Toenniessen GH (eds) Rice biotechnology. IRRI, Manila, pp 1–18

deOliveria CA, Sa NMH, Gomes EA et al (2009) Assessment of the mycorrhizal community in the rhizosphere of maize (*Zea mays* L.) genotypes contrasting for phosphorous efficiency in the acid savannas of Brazil using denaturing gradient gel electrophoresis (DGGE). Appl Soil Ecol 41:249–258

Dhillion SS (1992) Host Endophyte specificity of vesicular arbuscular mycorrhizal colonization of *Oryza sativa* L. at the pre-transplant stage in low or high phosphorus soil. Soil Biol Biochem 24:405–411

Dhillion SS, Ampornpan L (1992) Influence of inorganic fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant *Oryza sativa* L. plants. Biol Fert Soils 13:85–91

Duan M, Sun Z, Shu L, Tan Y, Yu D, Su X, Liu R, Li X, Gong S, Yuan D (2013) Genetic analysis of an elite super-hybrid rice parent using high density SNP markers. Rice 6:21

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 Agri Sci Camb 137:27–36

Edixhoven JD, Gupta J, Savenije HHG (2013) Recent revisions of phosphate rock reserves and resources: reassuring or misleading? An in-depth literature review of global estimates of phosphate rock reserves and resources. Earth Syst Dyn Discuss 4:1005–1034

Evans DG, Miller MH (1988) Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction of nutrients absorption in maize. 1. Causal relations. New Phytol 110:67–74

Foolad MR, Sharma A (2005) Molecular markers as selection tools in tomato breeding. Acta Hortic (695):225–240

Frank B (1885) Ueber die auf Wurzelsymbiose beruhende Ern¨ahrung gewisser B¨aume durch unterirdische Pilze. Ber Dtsch Bot Ges 3:128–145

Franken P, Requena N (2001) Analysis of gene expression in arbuscular mycorrhizas: new approaches and challenges. New Phytol 150:517–523

Friberg S (2001) Distribution and diversity of arbuscular mycorrhizal fungi in traditional agriculture on the Niger inland delta, Mali, West Africa. CBM: s Srifteserie 3:53–80

Galvàn GA, Kuyper TW, Burger K, Keizer LCP, 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. https://doi.org/10.1007/s00122-010-1501-8

Gangopadhyay S, Das KM (1982) Occurrences of vesicular arbuscular mycorrhizae in India. Indian Phytopathol 35:83–85

Gao X, Kuyper TW, Zou C, Zhang F, Hoffland E (2007) Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when non-mycorrhizal. Plant Soil 290:283–291

Garciaromera I, Ocampo JA (1988) Effect of the herbicide MCPA on VA mycorrhizal infection and growth of *Pisum sativum*. J Plant Nutr Soil Sci 151:225–228

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agri Ecosyst Environ 113:17–35

Grant C, Bittman S, Montreal M, Plenchette C, Morel C (2005) Soil and Fertilizer phosphorus: effects on plant P supply and mycorrhizal development. Can J Plant Sci 85:3–14

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

Hetrick BAD, Wilson GWT, Todd TC (1992) Mycorrhizal dependence of modern wheat cultivars and ancestors. Can J Bot 70:2032–2040

Hetrick BAD, Wilson GWT, Todd TC (1993) Mycorrhizal dependence of modern wheat cultivars and ancestors: a symbiosis. Can J Bot 71:512–518

Hetrick BAD, Wilson GWT, Gill BS, Cox TS (1995) Chromosome location of mycorrhizal responsive genes in wheat. Can J Bot 73:891–897

Hetrick BAD, Wilson GWT, Todd TC (1996) Mycorrhizal response in wheat cultivars relationship to phosphorus. Can J Bot 74:19–25

Holford JCR (1997) Soil Phosphorus: its measurement and its uptake by plants. Aust J Soil Res 35:227–239

Hu W, Wen M, Han Z, Tan C, Xing Y (2013) Scanning QTLs for grain shape using a whole genome SNP array in rice. J Plant Biochem Physiol 1:104. https://doi.org/10.4172/2329-9029.1000104

International Rice Genome Sequencing Project (2005) The map sequence of the rice genome. Nature 436:793–800

Itao E, Ella E, Kawanto N (1999) Physiological basis of submergence tolerance in rainfed lowland rice ecosystem. Field Crop Res 64:75–90

Jackson ML (1971) Soil chemical analysis. Prentice Hall, New Delhi, p 574

Janos DP (1988) Mycorrhiza applications in tropical forestry: are temperate-zone approaches appropriate? In: NG FSP(Ed) trees and mycorrhiza. Forest Research Institute, Kuala Lumpur, pp 133–188

Janos DP (2007) Plant responsiveness to mycorrhiza differs from dependence upon mycorrhizas. Mycorrhiza 17:75–91
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
Johnson NC, Copeland PJ, Crookston RK, Pfleger FL (1992) Mycorrhizae: possible explanation for yield decline with continuous corn or soybean. Agron J 84:387–390
Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–585
Jones MD, Smith SE (2004) Exploring functional definition of mycorrhizas: are mycorrhizas always mutualisms? Can J Bot:1089–1109
Kaeppler 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
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 non-mycorrhizal crops. Plant Soil. 353:355–366
Kebriyaee D, Kordrostami M, Rezadoost MH, Lahiji HS (2012) QTL analysis of agronomic traits in rice using SSR and AFLP markers. Not Sci Biol 4(2):116–123
Kimani JM, Tongoona P, Derera J (2013) Breeding dynamics of rice (*Oryza sativa*) for enhanced adaptation and grain quality. Sci Res Essays 8:1258–1272
Knapp SJ (1991) Using molecular markers to map multiple quantitative trait loci: models for backcross. Recombinant inbred lines, and double haploid progeny. Theor Appl Genet 81:333–338
Koide RT (1991a) Tansley Review no. 29. Nutrient demand and plant response to mycorrhizal infection. New Phytol 117:365–386
Koide R (1991b) 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 bicolour* (L.) Moench. DNA simple sequence repeats (SSRs). Theor Appl Genet 101:438–448
Kumar SG, Kumari KA, Rani VD, Sundaram RM, Vanisree S, Jamaloddin M, Swathi G (2013) Study of simple sequence repeat (SSR) polymorphism for biotic stress polymorphism for biotic stress resistance in elite rice variety JGL 1798. Afr J Biotech 12:5833–5838
Langridge P, Chalmer K (2004) The principle: identification and application of molecular markers. In: Lorz H, Wenzel G (eds) Molecular marker systems in plant breeding and crop improvement, vol 55. Springer, Berlin, pp 3–22
Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. Mol Breed 1:389–395
Leiser WL, Olatoye MO, Rattunde FW, Neumann G, Weltzien E, Haussman BIG (2016) No need to breed for enhanced colonization by arbuscular mycorrhizal fungi to improve low-P adaptation of West African Sorghums. Plant Soil 401:51–64
Li Y, Tao H, Xu J, Shi Z, Ye W, Wu L, Zing D, Gao Z, Guo L (2015) QTL analysis for cooking traits of super rice with a high density SNP genetic map and fine mapping of a novel boiled grain length locus. Plant Breed 134:535–541
Lynch JP (2007) Roots of the second green revolution. Austrian J Bot 55:491–501
Maiti D (2011) Improving activity of native arbuscular mycorrhizal fungi (AMF) for mycorrhizal benefits in agriculture: status and prospects. J Biofertil Biopestic 2:113. https://doi.org/10.4172/2155-6201.S1-001
Maiti D (2015) Improving phosphorus nutrition of upland rice through native arbuscular mycorrhiza (AM). J Rice Res 3:e115
Maiti D, Barnwal MK (2012) Optimization of phosphorus level for effective arbuscular-mycorrhizal activity in rainfed upland rice based cropping system. Indian Phytopathol 65:334–339

Maiti D, Variar M, Saha J (1995) Colonization of upland rice by native VAM under rainfed monocropped ecosystem. In: Roy AK, Sinha KK (eds) Recent advances in phytopathological research. M.D Publications, New Delhi, pp 45–52

Maiti D, Barnwal MK, Singh RK, Rana SK, Variar M (2006) Enhancing native arbuscular mycorrhizal association to improve phosphorus nutrition of rainfed upland rice (*Oryza sativa* L.) through cropping systems. Indian Phytopathol 59:432–438

Maiti D, Barnwal MK, Singh RK, Rana SK (2007) Approaches to utilize native arbuscular mycorrhizal association for improving P nutrition in upland rice under rainfed ecosystem. In: The mycorrhizae: diversity, ecology and applications. Daya, New Delhi, pp 193–199

Maiti D, Barnwal MK, Singh RK (2008) Exploring possibility of utilizing native arbuscular mycorrhizal fungi for improving phosphorus nutrition in transplanted rice (*Oryza sativa* L.) of Plateau region. Indian Phytopathol 61:302–304

Maiti D, Barnwal MK, Singh RK, Variar M (2009) A new protocol for on-farm production of arbuscular mycorrhizal mass inoculum for rainfed upland rice. Indian Phytopathol 62:31–36

Maiti D, Variar M, Singh RK (2011a) Optimizing tillage schedule for maintaining activity of the arbuscular mycorrhizal fungal population in rainfed upland rice (*Oryza sativa* L.) agroecosystem. Mycorrhiza 21:167–171

Maiti D, Toppo NN, Variar M (2011b) Integration of crop rotation and arbuscular mycorrhizal (AM) fungal inoculum application for enhancing native AM activity to improve phosphorus nutrition of upland rice (*Oryza sativa* L.) Mycorrhiza 21:659–667

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 Fertil Soils 48:67–73

Mararthi 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. https://doi.org/10.1186/1471-2229-12-137

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

McCouch SR, Zhao K, Wright M, Tung C, Ebana K, Thomson M, Reynolds A, Wang D, Declerck G, Ali L, Mclung A, Eizeng G, Bustamante C (2010) Development of genome wide SNP arrays for rice. Breed Sci 60:524–535

McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol Res 94:120–122

Menéndez 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

Mengel K, Kirby EA (2001) Principles of plant nutrition, 5th edn. Kluwer, Dordrecht

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance gene by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Miles C, Wayne M (2008) Quantitative trait locus (QTL) analysis. Nat Educ 1(1):208

Mosse B (1973) Advances in the study of vesicular-arbuscular mycorrhiza. Annu Rev Phytopathol 11:171–196

Muok BO, Matsumura A, Ishii T, Odee DW (2009) The effect of intercropping *Sclerocarya birrea* (A. Rich) Hochst., millet and corn in the presence of arbuscular mycorrhizal fungi. Afr J Biotechnol 8:807–812

Nosengo N (2003) Fertilized to death. Nature 425:894–895

Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemken A (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. Appl Environ Microbiol 69:2816–2824

Pearson JN, Jakobsen I (1993) The relative contribution of hyphae and roots to phosphorous uptake by arbuscular mycorrhizal plants measured by dual labelling with ^{32}P and ^{33}P. New Phytol 124:489–494

Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotrophism. Biosystems 6:153–164

Ramaekers L, Remans R, Rao IM, Blair MW, Vanderleyden J (2010) Strategies for improving phosphorous acquisition efficiency of crop plants. Field Crops Res 117:169–176

Rana SK, Maiti D, Barnwal MK, Singh RK, Variar M (2002) Effect of rice (*Oryza sativa* L.)-based cropping systems on vesicular arbuscular mycorrhizal colonization, P uptake and yield. Indian J Agric Sci 72:400–403

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

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci USA 91:11841–11843

Rose TJ, Impa SM, Rose MT, Pariasca-Tanaka J, Mori A, Heuer S, Johnson-Baebout SE, Wissuwa M (2013) Enhancing phosphorous and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. Ann Bot 112:331–345

Ryan MH, Graham JH (2002) Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant Soil 250:225–239

Saha R, Saha J, Bhattacharya PM, Maiti D, Chaudhury S (2005) Arbuscular mycorrhizal responsiveness of two rice varieties in nutrient deficient laterite soil. In: Prakash A, Mehrotra VS (eds) Mycorrhizae. V.S. Scientific, Jodhpur, India, pp 21–25

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

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. https://doi.org/10.1186/1471-2156-14-104

Sawers RJ, Gutjahr C, Paszkowski U (2008) Cereal mycorrhiza: an ancient symbiosis in modern agriculture. Trends Plant Sci 13:93–97

Schenck NC, Kinloch RA (1980) Incidence of mycorrhizal fungi on six in monoculture on a newly cleared woodland site. Mycologia 72:445–455

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

Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with land plants. Nature 363:67–69

Singh MP (2009) Rice productivity in India under variable climates. Paper presented at MARCO (Monsoon Asia Agro-Environmental Research Consortium) symposium, October 6–9, Tsukuba, Japan. Workshop 2 (October 6)

Sinha S (2011) Saturation mapping in-silico and association mapping of molecular markers with QTLs associated with drought tolerance in rice. PhD thesis, Dept. of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India

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, London

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

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

Smith FA, Smith SE (2012) Fresh perspectives on the roles of arbuscular mycorrhiza fungi in plant nutrition and growth. Mycologia 104:1–13

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plant irrespective of growth responses. Plant Physiol 133:16–20

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorous nutrition: interactions between pathways of phosphorous uptake in arbuscular mycorrhiza roots have important implications for understanding and manipulating plant phosphorous acquisition. Plant Physiol 156:1050–1057

Solaiman MZ, Hirata H (1997) Responses of directly seeded wetland rice to arbuscular mycorrhizal fungi inoculation. J Plant Nutr 20:1479–1487

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Tawaraya 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

Thompson JP (1991) Improving the mycorrhizal condition of the soil through cultural practices and effects on growth and phosphorus uptake in plants. In: Johansen C, Lee KK, Sahrawat KL (eds) Phosphorus nutrition of grain legumes in the semi-arid tropics. ICRISAT, Patancheru, India, pp 117–137

Toppo NN, Maiti D, Srivastava AK (2012) Native arbuscular mycorrhizal fungal diversity in rice based cropping systems under rainfed ecology. Columban J Life Sci 13:79–85

Toppo NN, Srivastava AK, Maiti D (2013) Effect of arbuscular mycorrhizal (AM) inoculation on upland rice root system. Bioscan 8:533–536

Toppo NN, Jambhulkar NN, Nitin M, Srivastava AK, Maiti D (2016) On-farm production of vermiculite based indigenous AM fungus inoculum for improved phosphorous nutrition in upland rice. Ecol Environ Conservat 22:203–211

Turrini A, Giordani T, Avio L, Natali L, Giovannetti M, Cavallini A (2016) Large variation in mycorrhizal colonization among wild accessions, cultivars and inbreds of sunflower (*Helianthus annuus* L.) Euphytica 207:331–342

Verbruggen E, WFM RLING, Gamper HA, Kowalchuk GA, Verhoef HA, Vander Heijden MGA (2010) Positive effects of organic farming on below ground mutualistic large scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytol 186:98–979

Vierheilig H, Garcia-Garrido JM, Wyss U, Piché Y (2000) Systemic suppression of mycorrhizal colonization of barley roots already colonized by AM fungi. Soil Biol Biochem 32:589–595

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

Wijerathna YMAM (2015) Marker assisted selection: biotechnology tool for rice molecular breeding. Adv Crop Sci Technol 3:187. https://doi.org/10.4172/2329-8863.1000187

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

Ye C, Argargose MA, Redona ED, Sierra SN, Laza MA, Dilla CJ, Mo Y, Thomson MJ, Chen J, Delavina CB, Diaz CQ, Hernandez JE (2011) Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. Plant Breed 131:33–41

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501

Yu H, Xie W, Li J, Zhou F, Zhang Q (2014) A whole genome SNP array (Rice 6K) for genomic breeding in rice. Plant Biotechnol J 12:28–37

Zhu YG, Smith SE, Barritt AR, Smith FA (2001) Phosphorous (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. Plant Soil 237:249–255

Zhu YG, Smith FA, Smith SE (2003) Phosphorous efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. Mycorrhiza 13:93–100

Chapter 4
Arbuscular Mycorrhizal Fungi and Heavy Metal Tolerance in Plants: An Insight into Physiological and Molecular Mechanisms

Neera Garg, Sandeep Singh, and Lakita Kashyap

Abstract Arbuscular mycorrhiza (AM) are the obligate symbiotic fungi which are integral part of plant roots systems and have been documented to ameliorate heavy metal (HM) stress. AM have the ability to impart tolerance by modulating various physiological, biochemical and molecular mechanisms in the root rhizosphere as well as within the plants. At physiological levels, AM immobilizes HMs in soil through binding of toxic ions to the cell wall components, secretes numerous organic acids in the rhizosphere such as citric, oxalic acid and a glycoprotein, glomalin, which act as a chelating agent and reduce metal uptake in the plants. At biochemical level, mycorrhizal hyphae enhance the uptake of nutrients which are otherwise unavailable to the plants and this AM induced improved nutrient acquisition is reported to enhance plant growth and reduce metal concentrations in the plant tissues, as a result of dilution effect. In addition to increased nutrient acquisition, AM also enhance the antioxidant defense responses to counteract HM induced oxidative stress. Furthermore, at molecular level, AM upregulates the genes responsible for nutrient uptake and stimulation of metallothioneins and phytochelatins synthesis resulting in HM sequestration in the host plant as well as in mycorrhizal structures. On the basis of available literature, this article summarizes various mechanisms modulated by AM fungi in imparting HM tolerance to the plants.

4.1 Introduction

Heavy metal (HM) stress in the soil has emerged as one of the most challenging environmental factors that negatively influence crop growth and production worldwide (Meier et al. 2012). HMs are non-biodegradable, persistent and naturally occurring chemical constituents with atomic density more than 4 g/cm^3 or five times or greater than water (Emamverdian et al. 2015). Increase in anthropogenic

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

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_4

Table 4.1 Major sources of heavy metal pollution and their permissible limits for human health

Pollutants	Rank according to priority list of hazardous substances (ATSDR 2015)	Major sources	Minimal risk levels (ATSDR 2015) for chronic durations (1 year or longer (mg/kg/day)	References
Arsenic	1	Pesticides, fungicides, metal smelters, wrong agricultural Practices, irrigating with As contaminated water	0.0003 mg/kg/day	Van et al. (2016), Dutta and Bandopadhyay (2016)
Mercury	3	Pesticides, batteries, paper industry	0.0002 mg/m^3	Cozzolino et al. (2016)
Cadmium	7	Welding, electroplating, pesticide, fertilizer, Cd and Ni batteries, nuclear fission plant	0.0001 mg/kg/day	Hu et al. (2016)
Chromium	17	Mines, mineral sources	0.0009 mg/kg/day	Panda and Choudhury (2005)
Zinc	75	Refineries, brass manufacture, metal plating, plumbing	0.3 mg/kg/day	Klimek (2012)
Copper	118	Mining, municipal sewage and pesticides	0.01 mg/kg/day	Chen et al. (2015)

Source: https://www.atsdr.cdc.gov/spl/#modalIdString_myTable2015

activities like mining, smelting, combustion of fossil fuels, excessive use of fertilizers, pesticides, herbicides, and improper disposal of sewage sludge have further elevated HM concentration in the biosphere (Table 4.1) (Singh et al. 2015). HMs such as Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr) and metalloid like Arsenic (As), are non-essential for plant growth and are highly toxic even at sub-optimal concentrations while others like Copper (Cu), Zinc (Zn) etc. are required by the plants in traces for various catalytic activities. However, even the essential metals become easily toxic when present at supra-optimal levels in soil (Fig. 4.1).

Phytotoxicity and phytoavailability of metal(loids) to the plants depends on physico-chemical properties of the metals, edaphic factors such as pH, metal concentration in soil, redox reactions and plant species (Kamal et al. 2010; Singh et al. 2015). Metals enter into plant roots either symplastically or through apoplastic movement depending upon the type and concentration of metals (https://www.drdarrinlew.us/metal-contaminated-2/factors-that-influence-metal-uptake.html). Some of the metal(loid)s are highly mobile and share similar transporter as that of essential elements. Arsenic is mostly available to plants in two forms, where *As V*

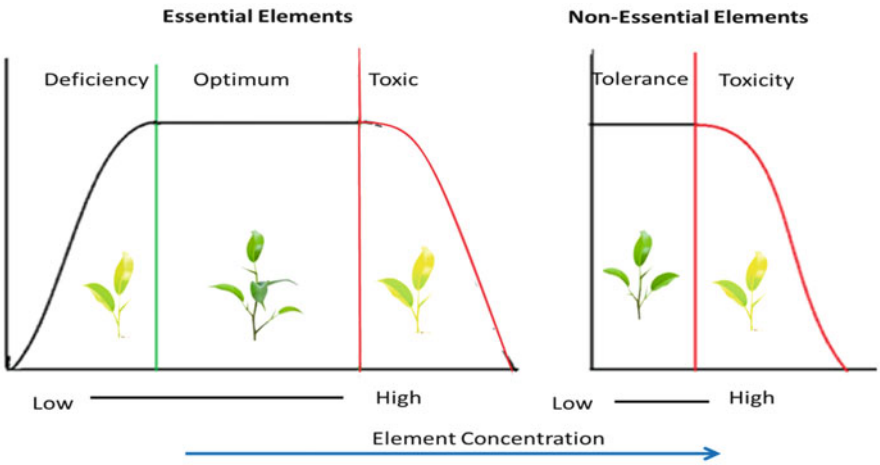


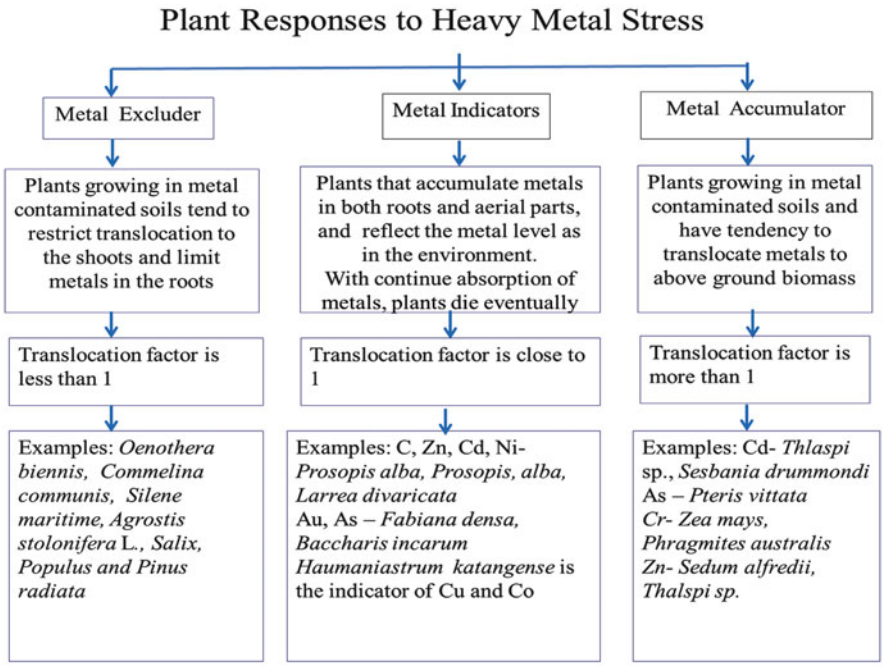
Fig. 4.1 Responses of plants to essential and non-essential elements in terms of their threshold limit

competes with Phosphorous transporters (Pht1) for uptake while in the form of *As III*, it is taken up by aquaporins (Verbruggen et al. 2009; Li et al. 2016). Cd is readily taken up via NRAMP (Natural Resistance-Associated Macrophage Proteins) and ZIP (Zinc Regulated Transporter/Iron Regulated Transporter) family of transporters (Takahashi et al. 2011; Wu et al. 2016) meant for uptake of Fe, Ca, Zn, and Mn (Clemens 2006; Gangwar et al. 2014). Hg is a class B metal which enters into the cells by competing with other metals like Cd and some essential metals such as Zn, Fe and Cu. Chromium (Cr) is a borderline toxic heavy metal existing in two stable forms i.e. *Cr III* and *Cr VI*. Plants uptake *Cr III* passively into the roots while *Cr VI* is actively taken up through Sulphur transporters (SULTR) (Oliveira 2012; Schiavon et al. 2012).

4.2 Strategies to Counteract HM Toxicity

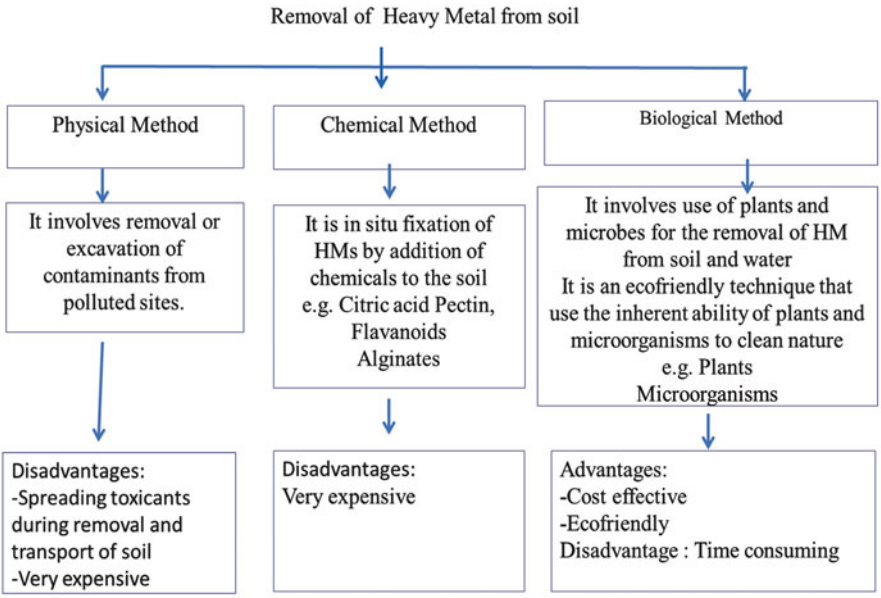
Elevated HM concentrations, whether essential or non-essential, generally have similar toxic effects on plants such as stunted plant growth, reduced plant biomass, distract nutrient acquisition, chlorosis, necrosis, altered water potential, senescence, ultimately leading to plant death (Gangwar et al. 2014; Singh et al. 2016). Toxic symptoms of different HMs might be due to alteration of membrane structure, replacement of an essential element from catalytic enzymes and binding with sulphydryl groups in proteins resulting into inactivation of enzyme activities (Capuana 2011). Furthermore, HMs are observed to overstimulate generation of reactive oxygen species (ROS) such as OH^-, O_2^- and H_2O_2 leading to production of oxidative stress in plants (Viehweger 2014) which disturbs cellular homeostasis,

membrane permeability and other metabolic functions, thereby adversely affecting growth and productivity (Li and Ramakrishna 2011; Aldoobie and Beltagi 2013). On the basis of their response to HM stress, plants have been categorized into three groups (Baker and Walker 1990; Mganga et al. 2011; Mehes-Smith et al. 2013) as follows:



The inherent strategies adopted by plants to counteract the detrimental effects of HM toxicity include avoiding exposure to toxic metals, minimizing uptake and internal compartmentalization of toxic ions (Ovečka and Takáč 2014). Possible primary detoxification mechanisms include secretion of exudates from roots, binding metals to the cell wall, active efflux, chelation with phytochelatins (PCs) and metallothioneins (MTs) and sequestration into vacuoles (Mishra and Dubey 2006; Hossain et al. 2012). Plants have evolved other mechanisms to counterbalance the ROS effects such as strengthening the activities of enzymatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APOX) and non-enzymatic antioxidants such as glutathione, ascorbate, and tocopherol (Dave et al. 2013). However, the upregulation of these tolerance mechanisms are often limited and vary from species to species depending upon their relative sensitivity to different concentrations of metal(loids).

Several techniques used for removal of HMs from contaminated sites include physical, chemical and biological processes:



4.3 Arbuscular Mycorrhizal Fungi: Potential Candidates for Bioremediation of Heavy Metal Stress

Among biological remediation processes, use of microorganisms like arbuscular mycorrhizal fungi (AMF) has gained importance in the recent years. AM fungi, belonging to the Glomeromycota, are ubiquitous rhizosphere micro flora forging symbiosis with the roots of 80–85% plants, including most agricultural, horticultural and hardwood crop species (Giovannetti et al. 2006; Berruti et al. 2016; Prasad et al. 2017). AMF play a major role in ecosystem functioning such as nutrient cycling, carbon sequestration, plant growth promotion by forming mutually beneficial associations in which plants receive nutrients like P, N in exchange of photosynthates required for the growth of mycorrhizal fungi (Kaiser et al. 2015). Mycorrhizal roots increase the root surface area due to their extramatrical hyphae, and therefore enable the plant to uptake water and nutrients (especially P) more efficiently from soil than non-mycorrhizal plants (Nadeem et al. 2014). According to Marschner and Dell (1994), AM fungus has the ability to supply about 80% of total phosphorus taken up by the mycorrhizal plants. In addition to increased P acquisition, AM also enhances uptake of other nutrients such as N, Mg, Cu, K and Zn, particularly in soils where they occur in less available forms (Clark and Zeto 1996; Smith and Read 2008). Transport of assimilates/nutrients from one plant to another through AM hyphal connections has been reported as mycorrhizal hyphae spread from roots of one infected plant to the roots of other nearby plants (Heap and

Newman 1980; Simard et al. 2012; Song et al. 2014). Moreover, they are considered highly beneficial to legume plant species through their synergistic and mutually beneficial interactions. Thus, this tripartite symbiotic relationship between legume–mycorrhiza–rhizobium results in enhanced N_2 fixing activity of rhizobia and improved overall plant growth (Barea et al. 2002; Jia et al. 2004; Wu et al. 2009). Mycorrhizal fungi also play a major role in soil aggregation through hyphal networking and production of various exudates like citric, malic, oxalic acids and a glycoprotein, glomalin (González-Chávez et al. 2004).

4.4 Occurrence of AMF in Heavy Metal Contaminated Soils

AMF species are capable of inhabiting extremely harsh environments, including HM polluted soils (Zarei et al. 2008; Cornejo et al. 2008; Miransari 2011). However, the diversity of AMF in these heavy contaminated soils is generally lower than in normal soils (Regvar et al. 2003; Hassan et al. 2011). In the contaminated soils, the number of mycorrhizal spores has been reported to strongly decrease, but the AMF propagules never disappear completely suggesting a certain adaptation of indigenous AMF to such environmental stresses (Zarei et al. 2008; Gamalero et al. 2009).

AMF have evolved various strategies to persist in HM contaminated environments and to avoid the damage produced by HMs (Fig. 4.2).

4.5 Mechanisms Adopted by AMF for HM Tolerance in Plants

4.5.1 Metal Avoidance

AMF is capable of altering its pattern of development in response to HM stresses where the mycelium tends to avoid metals by developing and exploring the unstressed parts of the environment (Pawlowska and Charvat 2004; González-Guerrero et al. 2008). Additionally, in order to help mycelium grow in metal stressed conditions, the spores and vesicles accumulate high metal contents than hyphae (González-Guerrero et al. 2008). AM fungal species have been reported to differ in their hyphal architecture, growth patterns and their tolerance level to HMs (Redecker and Raab 2006; Pawlowska et al. 1996; Ortega-Larrocea et al. 2001, 2007; Tonin et al. 2001; Vallino et al. 2006). Glomeraceae has a better potential to tolerate HMs (Öpik et al. 2006; Zarei et al. 2008), since they are capable of forming extensive hyphal network through crosslinks called anastomoses between the hyphae while other species like that of Gigasporaceae lacks such ability to

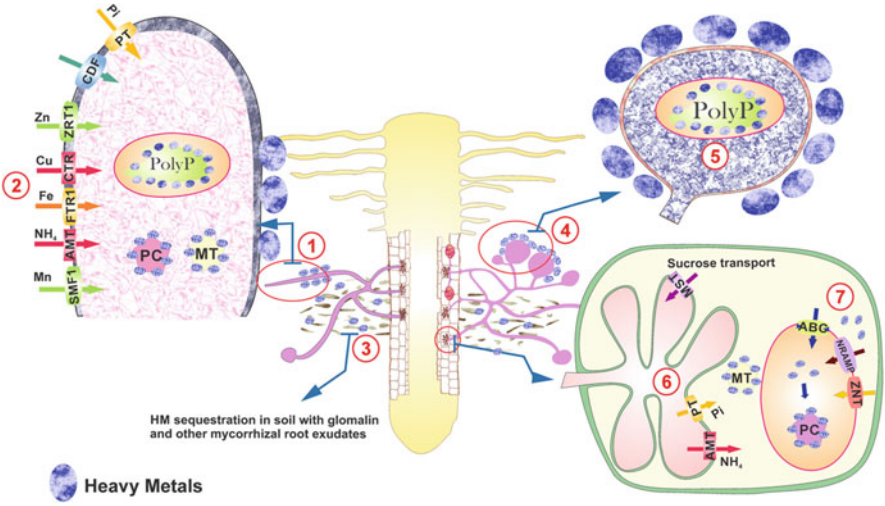


Fig. 4.2 Diagrammatic representation of mechanisms adopted by AM under HM stress: (1) Heavy metal adsorption on the surface of fungal hyphae. (2) Metal transporters located at the extra radicular mycelium (ERM) i.e. CDF, cation diffusion facilitator; CTR, fungal Cu transporter; FTR1, iron permesase; SMF1, Mn transporter; ZRT1, Zn transporter; PT, phosphate transporter; AMT, NH_4 transporter, contributing to enhanced nutrient acquisition, followed by sequestration of HMs in the hyphal cytoplasm through PCs and MTs and compartmentalization in the vacuoles by PolyP (PolyPhosphate) granules. (3) HM sequestration in soil through chelation by glomalin and other root/hyphal exudates. (4) HM immobilization on the surface of spore's cell wall. (5) HMs are stabilized through sequestration with PolyP in the vacuoles of the spores. (6) Nutrient exchange through arbuscules *via* transporters i.e. MST, Sucrose Transporter; PT and AMT on the surface of arbuscules. (7) HM stabilization in the vacuoles of plant cells through ABC, ABC transporter; NRAMP (natural resistance-associated macrophage proteins) family; MT, metallothionein; PC, phytochelatin; ZnT, Zn transporter

germinate from hyphae, to form extensive hyphae, and colonize roots only from germinating spores which are oriented towards the individual spreading of thick hyphae (Klironomos 2002; Voets et al. 2006). On the other hand few reports indicate occurrence of *Acaulospora*, *Scutellospora* and *Gigaspora* in moderate or highly HM polluted soils (Khan et al. 2000; Whitfield et al. 2004). The reason for predominance of *Glomus* species may be because of their more adaptability and higher sporulation rate (Daniell et al. 2001), through which it can infect and colonize the plant roots more rapidly and enhance the uptake of nutrients and water to plants (de la Providencia et al. 2005; Voets et al. 2006; Jansa et al. 2008) as compared to other families of AMF under stress conditions (Evelin et al. 2009; Krishnamoorthy et al. 2015). In addition, *Glomus* species have a different life cycle strategy, as they can not only be propagated by spores, but also by the hyphae residuals and mycorrhizal roots, while the other species germinate from their large and relatively sensitive spores (Franken and George 2006). In addition, the ability of *Glomus* species to form presymbiotic spores (secondary spores) allows the dispersal of these species in instances when germination of primary spores fails

to establish an association with a host plant (Warner and Mosse 1980; Pawlowska and Charvat 2004). *G. intraradices* has been shown to produce presymbiotic spores under Cd and Pb concentrations (Pawlowska and Charvat 2004).

4.5.2 Enhanced Water and Nutrient Uptake

The interaction between HMs and plant root cells results in physiological alterations that are involved in membrane damage and reduced uptake of water and nutrients (Astolfi et al. 2012; Rizzardo et al. 2012). AM fungal symbiosis with the host root increase the plant root absorptive surface area due to extraradical fungal hyphae exploring larger areas beyond the root-hair zone. This enhances the uptake of water and mineral nutrients which results in greater biomass production under stressed conditions (Goltapeh et al. 2008; Upadhyaya et al. 2010). The most important role of AM is the uptake of inorganic phosphate (Pi), along with nitrogen (N) and other trace elements like Cu and Zn (Smith and Read 2008; Ferrol and Pérez-Tienda 2009). Nutrients absorbed by the hyphae from the soil are translocated to the plant-fungus interface where two way exchange of nutrients and carbohydrates takes place. AMF forms appressorium on the root epidermis from which hyphae penetrate into root cortex where it forms highly branched structure called as arbuscules (Gianinazzi-Pearson et al. 1996). Upregulation of membrane transporter genes have been reported in AM as well as in mycorrhizal plants (http://www.davidmoore.org.uk/assets/mostly_mycology/diane_howarth/nutrients.html). Genes encoding for transport proteins *GiPT, GmosPT and GvPT* that are involved in phosphate uptake have been identified in the extraradical mycelium of *G. intraradices* (Maldonado-Mendoza et al. 2001), *G. mosseae* (Benedetto et al. 2005) and *Glomus versiforme* (Harrison and van Buuren 1995) respectively. In addition, H^+-ATPases have been identified in *G. mosseae* which are responsible for Pi uptake across the plasma membrane of ERM hyphae (Ferrol et al. 2000; Requena et al. 2003). AM inducible Pi transporters have also been documented in various crop plants such as *H. vulgare*, *T. aestivum*, *S. lycopersicum*, *Lotus* and *Medicago etc.* (Javot et al. 2007; Grace et al. 2009). Andrade et al. (2004) reported higher ratios of P/metal in several mycorrhizal plant species, suggesting that the greater P status of these plants might be responsible for alleviating metal stress through complexation of metal ions with phosphate inside the cells. Garg and Singla (2012) observed enhanced N, P, K^+ contents in plants associated with *G. mosseae*, which lead to improved plant growth .In addition, higher N/Cd, P/Cd and S/Cd ratios have been reported in both shoots and roots of mycorrhizal maize plants (de Andrade and da Silveira 2008; Garg et al. 2015). This higher N and S uptake in shoot of mycorrhizal plants leads to higher production of thiol rich proteins which might help in HM complexation with thiol compounds (Kapoor et al. 2013). Three ammonium transporters i.e. GintAMT1, GintAMT2 (López-Pedrosa et al. 2006; Pérez-Tienda et al. 2011, 2012) and GintAMT3 (Calabrese et al. 2016) have been identified in *R. irregularis*. All the three transporters have

been reportedly expressed in extra and intra radicular mycelium (ERM and IRM) and participate in the uptake of NH_4^+ from the soil solution. AMF is also known to induce sulphur (S) transporters, LjSultr1, 2 which appears to encode a key protein involved in plant sulfate uptake which mediates both direct and symbiotic pathways of S uptake in *L. japonicas* (Giovannetti et al. 2014). Higher K^+ accumulation was reported in spores, hyphae and vesicles of *R. irregularis* which was evaluated by PIXE (reviewed by Garcia and Zimmermann 2014). Moreover, K^+ enrichment has also been observed in *Aster tripolium* (Scheloske et al. 2004), *Zea mays* (Kaldorf et al. 1999) and *Lactuca sativa* (Baslam et al. 2013) inoculated with mycorrhiza, suggesting increase of K^+ acquisition due to AM colonization. The active uptake of all these nutrients results in enhanced root and shoots growth with AMF and reduction in the toxicity of HM due to "dilution effect", a possible mechanism through which AMF improves tolerance to HM stress in host plants (Leyval et al. 1997). Chen et al. (2007) reported almost six times increased plant biomass by AMF for *P. vittata*, *C. drummondii* and *T. repens* due to dilution effect under Cu and Cd stress. Garg and Aggarwal (2011) observed improved growth in *G. mosseae* inoculated pigeonpea plants with a decline in Cd and Pb concentrations which could be a consequence of dilution effect. Spagnoletti and Lavado (2015) reported improved plant yield and dilution effect resulting in reduced As concentrations in mycorrhizal soybean plants On the other hand, Agely et al. (2005) observed increased frond dry mass and increased As uptake in hyperaccumulator *Pteris vittata* when inoculated with AMF. Turnau and Mesjasz-Przybylowicz (2003) found higher shoot biomass and higher Ni uptake in hyperaccumulating *Berkheya codii* plants inoculated with native AMF than non-inoculated plants in a greenhouse experiment.

4.5.3 Metal Adsorption/Chelation

AM fungi affect the metal uptake by plants and their translocation from roots to shoots. The uptake of HMs could be enhanced or reduced depending upon the type of HMs, plants and fungal species/isolates (Emamverdian et al. 2015). AMF generally have a strong influence on plant biomass in the latter case due to restricted uptake of HMs. Mycorrhiza develops different strategies to withstand HM stress as the possibilities for mycelium to avoid these toxic metals are very limited in highly contaminated sites. AMF secretes glomalin, an iron-containing glycol-soil-proteinaceous substance, which is involved in heavy metal inactivation (Rillig et al. 2003; Ferrol et al. 2009; Gamalero et al. 2009) through chelation of HMs in the soil (González-Chávez et al. 2004). González-Chávez et al. (2004) reported that glomalin can bind 4.3 mg Cu, 1.12 mg Pb and 0.08 mg Cd per gram of protein from polluted soils. Moreover, under in vitro conditions, glomalin, from hyphae of *Gigaspora rosea* isolates, sequestered up to 28 mg Cu g^{-1}. Similarly, Bedini et al. (2010) reported that glomalin bound 2.3, 0.83, 0.24, and 0.24% of the total content of Cu, Ni, Pb, and Co respectively. Glomalin thus, stabilizes the HMs by reducing

their availability in soil and decreasing risk of toxicity to other soil microorganisms and plants growing in these sites (Cornejo et al. 2008). Gonzalez-Chavez et al. (2002a) reported irreversible sequestration of metals such as Cu, Cd, Zn and As in glomalin extracted from polluted soil. Cornejo et al. (2008) reported abundance of a glomalin-related soil protein in Cu and Zn polluted soils which is responsible for their sequestration in the soil. Similar results were obtained by Vodnik et al. (2008) for Pb and Zn sequestration. Another strategy adopted by AM include immobilization of HMs in the soil by exudation of several compounds such as citrates and oxalates (Green and Clausen 2003). Several cell wall-binding molecules like chitin, glucan and galactosamine polymers, proteins and peptides have been reported on mycorrhizal hyphae which represent potential binding sites as free hydroxyl, phosphate, carboxyl and amino groups (Bellion et al. 2006). Galli et al. (1994) reported that cell wall components like chitin, cellulose, cellulose derivatives and melanins of mycorrhizal fungi bound most of the potentially toxic elements such as Cu, Pb, Cd, etc. (Kapoor and Viraraghavan 1995). Orlowska et al. (2008) reported high binding capacity of ERM for Zn, Ni and Cu in *Berkheya coddii* mycorrhizal *G. mosseae* plants. Similarly, Zn and Cu were also reported to be deposited in the cell wall of the root of mycorrhizal plants and AM fungal wall (Marques et al. 2007; Zhang et al. 2009).

4.5.4 Metal Sequestration

The AM fungi tend to sequester HMs in vacuoles and cytosol of hyphae and spores under high metal concentrations. Chen et al. (2001) reported concentrations of over 1200 mg kg^{-1} and 600 mg $kg^{-1}$of Zn in fungal tissues of *G. mosseae* and *G. versiforme* respectively. The sequestration of HMs in fungal tissues or spores is achieved through metal chelators such as organic acids, amino acids, glutathione, phytochelatins (PCs) and metallothioneins (MTs) (Stommel et al. 2001; Cobbett and Goldsbrough 2002). Metallothioneins, on the bases of cysteine residues arrangements, are classified into two classes (Kojima 1991); Class I MTs found in vertebrates, and class II MTs in plants and fungi). *GrosMT1*, *GmarMT1* and *GintMT1* are the genes encoding metallothioneins that have been identified in *Gigaspora rosea*, *G. margarita* and *G. intraradices* respectively under stressed as well as unstressed conditions (Stommel et al. 2001; Lanfranco et al. 2002; Gonzalez-Guerrero et al. 2007; Saraswat and Rai 2011). *GmarMT1*, a gene encoding MT was observed to express in presymbiotic spores as well as in symbiotic mycelia in *Gigaspora margarita* under unstressed conditions. However upregulation of *GmarMT1* was reported in symbiotic mycelia when exposed to Cu stress (Lanfranco et al. 2002). *GintMT*, MT gene, isolated from *G. intraradices* was observed to impart Cu tolerance in Cu-sensitive *Saccharomyces cerevisiae* (Gonzalez-Guerrero et al. 2007). Rivera-Becerril et al. (2005) reported several genes encoding enzymes responsible for PC synthesis in mycorrhizal pea plants under Cd stress. At the genetic level, few metal transporter genes involved in heavy

metal homeostasis have been analysed in AMF. Zn transporter *GintZnT1* from *Glomus intraradices have been* reportedly involved in Zn compartmentalization in vacuole (González-Guerrero et al. 2005). González-Guerrero et al. (2010) characterized an ABC transporter of the MRP subfamily, *GintABC1*, which might be responsible for Cu and Cd trafficking into the vacuole. Similarly, genes encoding putative transport proteins mediating the uptake of Cu, Fe and Mn have been identified in *R. irregularis*. These include RiCTR1 and RiCTR3 (CTR family of Cu transporters), RiZRT1 (zinc–iron permease or ZRT-IRT-like protein family), RiSMF1 (NRAMP- natural resistance-associated macrophage proteins family) and RiFTR1 (iron permease) (Tisserant et al. 2013; Tamayo et al. 2014; Ferrol et al. 2016). All these genes play an important role in metal loading into vacuoles. Under high HM concentrations, the metals in vacuoles are diverted to the spores (González-Guerrero et al. 2008), explaining the tolerance of mycorrhiza against HMs in soils (Ferrol et al. 2009). Similarly, the reduced uptake of As by AMF could be due to the involvement of AM in suppressing the high-affinity phosphate transporters which also uptake As (Gonzalez-Chavez et al. 2002b; Ultra et al. 2007; Christophersen et al. 2012; Chen et al. 2014). Burleigh et al. (2003) reported that mycorrhiza (*Glomus versiforme*) down-regulated the expression of MtZIP2 Zn transporter gene in the roots of *Medicago* plants which was associated with a reduced Zn level in the host tissues. According to Khan et al. (2000), Zn is absorbed and crystallized in AMF hyphae under high Zn concentrations. González-Guerrero et al. (2008) showed localization of toxic concentrations of Cu, Zn and Cd in the fungal cell wall and spores of *G. intraradices* using the Energy-dispersive X-ray spectroscopy.

However, stabilization of the HMs in fungal structures is not a universal phenomenon as opposite results in some cases were observed where AM inoculation lead to significantly increased HM translocation from roots to shoots of mycorrhizal plants. Most of the metals taken up by the fungus can be stored in the vesicles or vacuoles of fungal hyphae, while the surplus HMs transferred from the fungal mycelium to the plant. AM fungi have developed mechanism to sequester and deliver the HMs in a bound form as free HMs in the cytosol represent a serious threat to the organelles. For example, in the case of Cu, the Cu chaperones collect the metal from the influx transporters and deliver it to efflux transporters or to apometalloproteins. In *R. irregularis*, three genes *RiATOX1*, *RiSco1* and *RiSSC* encoding putative chaperones have been identified that deliver Cu to the ATPases, the cytochrome C oxidase synthesis, and the Cu, Zn superoxide dismutase respectively (Ferrol et al. 2016).

4.5.5 Antioxidant Defense System

Metals adversely affect cell integrity and functioning by altering plant metabolic activities including cell enzymatic processes. Exposure to HM stress enhances the production of reactive oxygen species (ROS) including O_2^-, H_2O_2 and OH^- and

their accumulation in the cells (Mittler 2002). Plants have inherent defense mechanisms that are actively involved in counteracting the oxidative stress damage induced by ROS (Foyer and Noctor 2011; Wu et al. 2014). There are numerous antioxidant enzymes formed in response to ROS generation including catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione peroxidase (GPX), etc. The activity of these antioxidants is often limited and varies within the plant species as well as in response to different HMs. Regulation of antioxidant enzyme activity in the *G. mosseae* inoculated pigeon pea plants under Zn stress appeared to be limited as AM plants showed increased activity of SOD enzyme, with no effect on the activities of APX, GR or CAT in the presence of Zn (Schützendübel and Polle 2002; Garg and Kaur 2013). On the other hand, enhanced SOD, CAT, POX, GR activity and high ratio of reduced to oxidized glutathione (GSH/GSSG) has been reported in Cd-stressed AM plants (Garg and Aggarwal 2011; Garg and Kaur 2013). Similarly, Shahabivand et al. (2016) reported increased plant dry mass and activities of CAT, APX and GST in maize inoculated with *F. mosseae* under Cd stress. Moreover, AM fungi have also been reported to reduce oxidative stress under As and Cu stress by enhancing the production of enzymatic as well as non-enzymatic enzymes such as SOD, CAT, APX and GR (Garg and Singla 2012; Pallara et al. 2013).

4.6 Interactions Between Arbuscular Mycorrrhiza and Other Soil Microflora

Presence of other soil microflora may have a positive, negative or no response to AMF (Amballa and Bhumi 2016). The interaction between AMF and other fungi, bacteria, archaea, virus and algae depends upon the physico-chemical properties of the soil, pH, types of plant species, exudates from plant roots, presence of soil organic matter and nematodes causing disturbance to the soil (Hinsinger et al. 2005; Hartmann et al. 2009). AMF inhabits generally in two entirely different types of environments i.e. (1) root cortex with more stable surrounding and less microbe diversity, (2) soil with diversity of microbes (Jansa and Gryndler 2010). Due to presence of reduced carbon (C), maximum microbial activities have been reported in the closest proximity of plant roots (Jones et al. 2009). Exudates from hyphae are utilized by microbes (Toljander et al. 2007), while on the other hand microbes provide AMF with the unavailable nutrients (Frey-Klett et al. 2007). AM fungi interact with other soil microorganisms directly by competition for nutrition and indirectly by alteration of root and soil structure (Wehner et al. 2010).

4.6.1 Positive Correlation

Positive interactions between AMF and plant PGPRs have been documented in numerous studies (Artursson et al. 2006; Trabelsi and Mhamdi 2013). AMF when inoculated in combination with phosphate solubilizing bacteria and *Azospirillum* affect the plant growth synergistically (Muthukumar et al. 2001). AMF and Rhizobium are able to get along with one another so well due to the reason that both microsymbionts induce common signaling cascade for association with host plant (Manchanda and Garg 2007). Dual application of *Trichoderma harzianum* and *G. mosseae* was documented to improve yield and seed quality of soyabean in sterilized and unsterilized soils (Egberongbe et al. 2010). Kawasaki et al. (2011) and Trabelsi and Mhamdi (2013) observed that some legumes manage to form additional association with AMF to counteract soil contamination by altering the quantity and composition of root exudates. Decline in nodulation and nitrogen fixation in faba beans was observed when inoculated with *R. leguminosarum* or AMF alone in alkaline soils. However, dual application of rhizobia and AMF mix (*G. gigaspora, G. mosseae, S. armeniaca*) improved the nodulation due to enhanced uptake of nutrients such as Fe which is required for biosynthesis of nitrogenase and leghemoglobin (Abd-Alla et al. 2014). Inoculation of pigeon pea genotypes with *G. mosseae* and *Sinorhizobium fredii* had a functional complementarity in improving nodulation potential, nitrogen fixation as well as trehalose turnover (Garg and Aggarwal 2011; Garg and Kaur 2012; Garg and Chandel 2015; Garg et al. 2015). *Bacillus circulans*, the phosphate solubilizing bacteria in combination with AMF improve the plant growth tremendously (Massoud et al. 2014). Positive interaction of AMF with phosphate solubilizing, nitrogen fixing, siderophores, chitinase producing and other free living bacteria leading to enhanced plant growth have been documented in numerous studies (Amballa and Bhumi 2016; Ma et al. 2016). Growth promoting bacteria such as *Pseudomonas* spp. *Bacillus* spp., *Paenibacillus* spp. increase plant growth directly by enhancing phosphorus and nitrogen bioavailability for plants and secretion of phytohormones (Ahemad and Kibert 2014) or indirectly by synthesis of antibiotics which are toxic to plant pathogens or production of siderophores (Amballa and Bhumi 2016).

4.6.2 Negative Correlation

Reports on the negative impacts (if any) of AMF on population of other soil microflora are scanty. Mycorrhiza in soil have been reported to protect plants from pathogen by releasing some toxins, providing mechanical strength to the roots, activating plant defense mechanisms such as stimulation of flavonoids salicylic acids, jasmonates and by alteration of soil microflora due to modification in root architecture and exudates (Tiberius and Cătălin 2011). AM has also been reported to have detrimental effects on some of the soil microorganisms due to

production of secondary metabolites which affect them selectively (Trotta et al. 1996; Bødker et al. 1998; Wamberg et al. 2003). Conidial germination of *Fusarium oxysporum* was inhibited by *G. intraradices* which could be due to the reason that AM manipulated the pH of growth medium (Filion et al. 1999). Marschner (1997) observed altered rhizosphere soil composition and reduced root exudation in the vicinity of *Capsicum annuum* inoculated with *G. derticola* and *G. intraradices*, thereby resulting into reduced population density of *Pseudomonas fluorescens*. On the other hand, some of the pathogenic fungi are reported to affect AMF negatively due to competition for photosynthates, infection sites and space within root cortex as well as in rhizosphere (Whipps 2004). Ravnskov et al. (1999) reported that *Pseudomonas putida* growth and survival was inhibited by *Glomus intraradices* under normal conditions due to competition for nutrition. Negative impact of mircroorganisms on AMF include decreased spore germination, reduced hyphal length and various metabolic activities in hyphal structures as well as inhibited mycorrhizal colonization (Amballa and Bhumi 2016). AMF generally have multiple trading partners by formation of common mycorrhizal networks for continuous supply of Carbon. These common mycorrhizal networks transfer information and warning signals between the plants. Common mycorrhizal networks transfer the allelochemicals secreted by plants to regulate the activity of competitive neighboring plants as well as other micro-organisms. Some of these allelochemicals have antifungal properties that suppress the mycorrhizal colonization in native competitors (Barto et al. 2012; Bucking et al. 2016).

4.7 Conclusion and Future Prospects

Mycorrhizal symbiosis plays an important role in shaping up soil ecosystems by promoting soil fertility and plant health. Presence of AM in metal rich soils indicates their adaptation to different HMs. However, understanding the mechanisms adopted by different AM fungi in the alleviation of HM toxicity in plants is complex and varies according to soil properties, plant species as well as the diversity of physiological and molecular mechanisms affecting plant-fungal symbiosis. The present review highlighted the role of AM fungi in modulating the soil characteristics in terms of immobilization/stabilization of HMs, reducing their uptake and translocation within the plant organ as well as metal sequestration into the vacuoles. Despite the identification of genes involved in fungal and plant perception, better understanding of molecular mechanisms and signaling pathways coupled to establishment of an effective AM-plant symbiosis need to be unraveled. Future studies should be directed in understanding the complexities of processes involved in soil/plant/microenvironment and the resultant impact on establishment and survival of different microbial communities.

References

Abd-Alla MH, El-Enany A-WE, Nafady NA, Khalaf D, Morsy FM (2014) Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol Res 169:49–58

Agely AL, Sylvia DM, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator chinese brake fern (*Pteris vittata* L.) J Environ Qual 34:2181–2218

Ahemad M, Kibert M (2014) Mechanisms and application of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Aldoobie NF, Beltagi MS (2013) Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. Afr J Biotechnol 12:4614–4622

Amballa H, Bhumi NR (2016) Significance of arbuscular mycorrhizal fungi and rhizosphere microflora in plant growth and nutrition. In: Devendra KC, Ajit V, Narendra T (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore, pp 417–452

Andrade SAL, Abreu CA, de Abreu MF, da Silveira APD (2004) Influence of lead addition on arbuscular mycorrhiza and *Rhizobium* symbioses under soybean plants. Appl Soil Ecol 26:123–131

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

Astolfi S, Zuchi S, Neumann G, Cesco S, di Toppi LS, Pinton R (2012) Response of barley plants to Fe deficiency and Cd contamination as affected by S starvation. J Exp Bot 63:1241–1250

ATSDR (2015) Agency for Toxic Substances and Disease Registry. Priority List of Hazardous Substances. https://www.atsdr.cdc.gov/spl/#modalIdString_myTable2015

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants: heavy metal tolerance in plants. In: Shaw AJ (ed) Evolutionary aspects. CRC, Boca Raton, pp 109–119

Barea JM, Toro M, Orozco MO, Campos E, Azcón R (2002) The application of isotopic (P_{32} and N_{15}) dilution techniques to evaluate the interactive effect of phosphate solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to improve the agronomic efficiency of rock phosphate for legume crops. Nutr Cycl Agroecosyst 63:35–42

Barto EK, Weidenhamer JD, Cipollini D, Rillig MC (2012) Fungal superhighways: do common mycorrhizal networks enhance below ground communication? Trends Plant Sci 17 (11):633–637

Baslam M, Garmendia I, Goicoechea N (2013) The arbuscular mycorrhizal symbiosis can overcome reductions in yield and nutritional quality in greenhouse-lettuces cultivated at inappropriate growing seasons. Sci Hortic 164:145–154

Bedini S, Turrini A, Rigo C, Argese E, Giovannetti M (2010) Molecular characterization and glomalin production of arbuscular mycorrhizal fungi colonizing a heavy metal polluted ash disposal island, downtown Venice. Soil Biol Biochem 42:758–765

Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181

Benedetto A, Magurno F, Bonfante P, Lanfranco L (2005) Expression profiles of a phosphate transporter gene (*GmosPT)* from the endomycorrhizal fungus *Glomus mosseae*. Mycorrhiza 15:620–627

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. https://doi.org/10.3389/fmicb.2015.01559

Bødker L, Kjoller R, Rosendahl S (1998) Effect of phosphate and the arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza 8:169–174

Bucking H, Mensah JA, Fellbaum CR (2016) Common mycorrhizal networks and their effect on the bargaining power of the fungal partner in the arbuscular mycorrhizal symbiosis. Commun Integr Biol 9:e1107684

Burleigh SH, Kristensen BK, Bechmann IE (2003) A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol Biol 52(5):1077–1088

Calabrese S, Pérez-Tienda J, Ellerbeck M, Arnould C, Chatagnier O, Boller T, Courty P-E (2016) GintAMT3—a low-affinity ammonium transporter of the arbuscular mycorrhizal *Rhizophagus irregularis*. Front Plant Sci 7:679

Capuana M (2011) Heavy metals and woody plants-biotechnologies for phytoremediation. Biogeosci For 4:7–15

Chen B, Christie P, Li L (2001) A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. Chemosphere 42:185–192

Chen BD, Zhu Y-G, Duan J, Xiao XY, Smith SE (2007) Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environ Pollut 147:374–380

Chen A, Chen X, Wang H, Liao D, Gu M, Qu H, Sun S, Xu G (2014) Genome-wide investigation and expression analysis suggest diverse roles and genetic redundancy of Pht1 family genes in response to Pi deficiency in tomato. BMC Plant Biol 14:61

Chen J, Shafi M, Li S, Wang Y, Wu J, Ye ZQ, Peng DL, Yan WB, Liu D (2015) Copper induced oxidative stresses, antioxidant responses and phytoremediation potential of Moso bamboo (*Phyllostachys pubescens*). Sci Rep 5:13554

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. https://doi.org/10.3389/fphys.2012.00091

Clark RB, Zeto SK (1996) Iron acquisition by mycorrhizal maize grown on alkaline soil. J Plant Nutr 19:247–264

Clemens S (2006) Toxic metal accumulation responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719

Cobbett C, Goldsbrough PB (2002) Phytochelatins and metallothioneins roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cornejo P, Meier S, Borie G, Rillig M, Borie F (2008) Glomalin related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci Total Environ 406:154–160

Cozzolino V, De Martino A, Nebbioso A, Di Meo V, Salluzzo A, Piccolo A (2016) Plant tolerance to mercury in a contaminated soil is enhanced by the combined effects of humic matter addition and inoculation with arbuscular mycorrhizal fungi. Environ Sci Pollut Res 23:11312–11322

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonizing arable crops. FEMS Microbiol Ecol 36:203–209

Dave R, Tripathi RD, Dwivedi S, Tripathi P, Dixit G, Sharma YK, Trivedi PK, Corpas FJ, Barroso JB, Chakrabarty D (2013) Arsenate and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic accumulating rice (*Oryza sativa* L.) genotypes. J Hazard Mater 262:1123–1131

de Andrade SAL, da Silveira APD (2008) Mycorrhiza influence on maize development under Cd stress and P supply. Braz J Plant Physiol 20(1):39–50

de La Providencia IE, De Souza FA, Fernandez F, Séjalon-Delmas N, Declerck S (2005) Arbuscular mycorrhizal fungi exhibit distinct pattern of anastomoses formation and hyphal healing mechanism between different phylogenic groups. New Phytol 165:261–271

Dutta P, Bandopadhyay P (2016) Arsenic pollution in agriculture: its uptake and metabolism in plant system. Agric Res Technol 1(5):ARTOAJ.MS.ID.555573

Egberongbe HO, Akintokun AK, Babalola OO, Bankole MO (2010) The effect of *Glomus mosseae* and *Trichoderma harzianum* on proximate analysis of soybean (*Glycine max* (L.) Merrill.) seed grown in sterilized and unsterilized soil. JARED 4(2):54–58

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:756120

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Ferrol N, Pérez-Tienda J (2009) Coordinated nutrient exchange in arbuscular mycorrhiza. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearsonet V (eds) Mycorrhizas—functional processes and ecological impact. Springer, Berlin, pp 7–87

Ferrol N, Barea JM, Azcón-Aguilar C (2000) The plasma membrane H+ -ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*. Curr Genet 37:112–118

Ferrol N, Gonzalez-Guerrero M, Valderas A, Benabdallah K, Azcon-Aguilar C (2009) Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. Phytochem Rev 8:551–559

Ferrol N, Tamayo E, Vargas P (2016) The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J Exp Bot (22):6253–6265. https://doi.org/10.1093/jxb/erw403

Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Franken P, George E (2006) Diversity of arbuscular mycorrhizal fungi. In: Benckiser G, Schnell S (eds) Biodiversity in agricultural production system. CRC, Boca Raton, pp 189–203

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Galli U, Schuepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plantarum 92:364–368

Gamalero E, Berta G, Glick BR (2009) The use of microorganisms to facilitate the growth of plants in saline soils. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, pp 1–22

Gangwar S, Singh VP, Tripathi DK, Chauhan DK, Prasad SM, Maurya JN (2014) Plant responses to metal stress: the emerging role of plant growth hormones in toxicity alleviation. In: Ahmad P, Rasoo S (eds) Emerging technologies and management of crop stress tolerance, vol 2. Academic, Elsevier, San Diego, CA, pp 215–248

Garcia K, Zimmermann SD (2014) The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci 5:337

Garg N, Aggarwal N (2011) Effects of interactions between cadmium and lead on growth, nitrogen fixation, phytochelatin, and glutathione production in mycorrhizal *Cajanus cajan* (L.) Millsp. J Plant Growth Regul 30(3):286–300

Garg N, Chandel S (2015) Role of arbuscular mycorrhiza in arresting reactive oxygen species (ROS) and strengthening antioxidant defense in *Cajanus cajan* (L.) Millsp. nodules under salinity (NaCl) and cadmium (Cd) stress. Plant Growth Regul 75:521–534

Garg N, Kaur H (2012) Influence of zinc on cadmium-induced toxicity in nodules of pigeonpea (*Cajanus cajan* L. Mill sp.) inoculated with arbuscular mycorrhizal (AM) fungi. Acta Physiol Plant 34:1363–1380

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 199(2):118–133

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

Garg N, Singla P, Bhandari P (2015) Metal uptake, oxidative metabolism, and mycorrhization in pigeonpea and pea under arsenic and cadmium stress. Turk J Agric For 39(2):234–250

Gianinazzi-Pearson V, Dumas-Gaudot E, Gollotte A, Alaoui AT, Gianinazzi S (1996) Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol 133: 45–57. https://doi.org/10.1111/j.1469-8137.1996.tb04340.x

Giovannetti M, Avio L, Fortuna P, Pellegrino E, Sbrana C, Strani P (2006) At the root of the wood wide web: self-recognition and non-self-incompatibility in mycorrhizal networks. Plant Signal Behav 1:1–5

Giovannetti M, Tolosano M, Volpe V, Kopriva S, Bonfante P (2014) Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in *Lotus japonicus*. New Phytol 204:609–619

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

Gonzalez-Chavez C, D'Haen J, Vangronsveld JJ, Dodd JC (2002a) Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. Plant Soil 240:287–297

Gonzalez-Chavez C, Harris PJ, Dodd J, Meharg AA (2002b) Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. New Phytol 15:163–171

González-Chávez MC, Carrillo-Gonzalez R, Wright SF, Nichols K (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323

González-Guerrero M, Azcón-Aguilar C, Mooney M, Valderas A, Mac Diarmid CW, Eide DJ, Ferrol N (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130–140

Gonzalez-Guerrero M, Cano C, Azcon-Aguilar C, Ferrol N (2007) GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17:327–335

González-Guerrero M, Melville L, Ferrol N, Lott JNA, Azcón-Aguilar C, Peterson RL (2008) Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal *Glomus intraradices*. Can J Microbiol 54:103–110

González-Guerrero M, Benabdellah K, Valderas A, Azcón-Aguilar C, Ferrol N (2010) GintABC1 encodes a putative ABC transporter of the MRP subfamily induced by Cu, Cd, and oxidative stress in *Glomus intraradices*. Mycorrhiza 20:137–146

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

Green F, Clausen CA (2003) Copper tolerance of brown-rot fungi: time course of oxalic acid production. Int Biodeterior Biodegrad 51:145–149

Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257

Hassan SD, Boon E, St-Arnaud M, Hijri M (2011) Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Mol Ecol 20:3469–3483

Heap AJ, Newman EI (1980) The influence of vesicular-arbuscular mycorrhizas on phosphorus transfer between plants. New Phytol 85:173–179

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168:293–303

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:872875

Hu Y, Cheng H, Tao S (2016) The challenges and solutions for cadmium-contaminated rice in China: a critical review. Environ Int 92–93:515–532

Jansa J, Gryndler M (2010) Biotic environment of the arbuscular mycorrhizal fungi in soil. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 209–236

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol 177:779–789

Javot H, Pumplin N, Harrison MJ (2007) Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. Plant Cell Environ 30:310–322

Jia Y, Gray VM, Straker CJ (2004) The influence of rhizobium and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. Ann Bot 94:251–258

Jones DL, Nguyen C, Finlay RD (2009) carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil 321:5–33

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

Kaldorf M, Kuhn AJ, Schroder WH, Hildebrandt U, Bothe H (1999) Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. J Plant Physiol 154:718–728

Kamal S, Prasad R, Varma A (2010) Soil microbial diversity in relation to heavy metals. In: Sherameti I, Varma A (eds) Soil heavy metals. Springer, Berlin, pp 31–64

Kapoor A, Viraraghavan T (1995) Fungal biosorption—an alternative treatment option for heavy metal bearing wastewater: a review. Biores Technol 53:195

Kapoor R, Evelin H, Mathur P, Giri B (2013) Arbuscular mycorrhiza: approaches for abiotic stress tolerance in crop plants for sustainable agriculture. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer, New York, pp 359–401

Kawasaki A, Watson ER, Kertesz MA (2011) Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizospheres. Plant Soil 358:1–14

Khan AG, Kuek C, Chaudhary TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Klimek B (2012) Effect of long-term zinc pollution on soil microbial community resistance to repeated contamination. Bull Environ Contam Toxicol 88(4):617–622. https://doi.org/10.1007/s00128-012-0523-0

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70

Kojima Y (1991) Definition and nomenclature of metallothioneins. Methods Enzymol 205:8–10

Krishnamoorthy R, Kim C-G, Subramanian P, Kim K-Y, Selvakumar G, Sa T-M (2015) Arbuscular mycorrhizal fungi community structure, abundance and species richness changes in soil by different levels of heavy metal and metalloid concentration. PLoS One. https://doi.org/10.1371/journal.pone.0128784

Lanfranco L, Bolchi A, Ros S, Ottonello S, Bonfante P (2002) Differential expression of metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Li K, Ramakrishna W (2011) Effect of multiple metal resistant bacteria from contaminated lake sediments on metal accumulation and plant growth. J Hazard Mater 15:531–539

Li N, Wang J, Song W-Y (2016) Arsenic uptake and translocation in plants. Plant Cell Physiol 57:4–13

López-Pedrosa A, González-Guerrero M, Valderas A, Azcón-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

Ma Y, Oliveira RS, Freitas H, Zhang C (2016) Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. Front Plant Sci 7:918

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ (2001) A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol Plant Microbe Interact 14:1140–1148

Manchanda G, Garg N (2007) Endomycorrhizal and rhizobial symbiosis: how much do they share? J Plant Inter 2:79–88

Marques APGC, Oliveira RS, Samardjieva KA, Pissara J, Rangel AOSS, Castro PML (2007) *Solanum nigrum* grown in contaminated soil: effect of arbuscular mycorrhizal fungi on zinc accumulation and histolocalisation. Environ Pollut 145:691–699

Marschner H (1997) Mineral nutrition of higher plants. Academic, London, p 889

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Massoud ON, Morsy EM, Bishara MM (2014) The promotive effect of N2 fixers, *Bacillus circulans* and *Saccharomyces cerevisiae* on the viability of native arbuscular mycorrhizal fungi and the impact on the productivity of alfalfa (*Medicago sativa* L.) Afr J Online 39:127–139

Mehes-Smith M, Nkongolo K, Cholewa E (2013) Coping mechanisms of plants to metal contaminated soil. In: Silvern S, Young S (eds) Environmental change and sustainability. InTech Open, Rijeka, pp 53–90

Meier S, Borie F, Bolan N, Cornejo P (2012) Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Crit Rev Environ Sci Technol 42:741–775

Mganga N, Manoko MLK, Rulangaranga ZK (2011) Classification of plants according to their heavy metal content around North Mara Gold Mine, Tanzania: implication for phytoremediation. Tanz J Sci 37:109–119

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653

Mishra S, Dubey RS (2006) Heavy metal uptake and detoxification mechanisms in plants. Int J Agric Res 1:122–141

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410

Muthukumar T, Udaiyan K, Manian S (2001) Vesicular-arbuscular mycorrhizal association in the medicinal plants of Maruthamalai Hills, Western Ghats, southern India. J Mycol Plant Pathol 31:180–184

Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32:429–448

Oliveira H (2012) Chromium as an environmental pollutant: insights on induced plant toxicity. J Bot 2012:375843

Öpik 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

Orlowska E, Mesjasz-Przybylowicz J, Przybylowicz W, Turnau K (2008) Nuclear macroprobe studies of elemental distribution in mycorrhizal and non-mycorrhizal roots of Ni-hyperaccumulator *Berkheya coddii*. X Ray Spectrom 37:129–132

Ortega-Larrocea MP, Siebe C, Becard G, Mendez I, Webster R (2001) Impact of a century of wastewater irrigation on the abundance of arbuscular mycorrhizal spores in the soil of the Mezquital Valley of Mexico. App Soil Ecol 16:149–157

Ortega-Larrocea MP, Siebea CA, Websterc ER (2007) Mycorrhizal inoculum potential of arbuscular mycorrhizal fungi in soils irrigated with wastewater for various lengths of time, as affected by heavy metals and available P. App Soil Ecol 37:129–138

Ovečka M, Takáč T (2014) Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnol Adv 32:73–86

Pallara G, Todeschini V, Lingua G, Camussi A, Racchi ML (2013) Transcript analysis of stress defence genes in a white poplar clone inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and grown on a polluted soil. Plant Physiol Biochem 63:131–113

Panda SK, Choudhury S (2005) Chromium stress in plants. Braz J Plant Physiol 17:95–102

Pawlowska TE, Charvat I (2004) Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Appl Environ Microbiol 70:6643–6649

Pawlowska TE, Blaszkowski J, Ruhling A (1996) The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. Mycorrhiza 6:499–505

Pérez-Tienda J, Testillano PS, Balestrini R, Fiorilli V, Azcón-Aguilar C, Ferrol N (2011) GintAMT 2, a new member of the ammonium transporter family in the arbuscular mycorrhizal fungus *Glomus intraradices*. Fungal Genet Biol 48:1044–1055

Pérez-Tienda J, Valderas A, Camañes G, García-Agustín P, Ferrol N (2012) Kinetics of NH_4^+ uptake by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Mycorrhiza 22:485–491

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

Ravnskov S, Nybroe O, Jakobsen I (1999) Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. New Phytol 142:113–122

Redecker D, Raab P (2006) Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. Mycologia 98:885–895

Regvar M, Vogel K, Irgel N, Wraber T, Hildebrandt U, Wilde P, Bothe H (2003) Colonization of pennycresses (*Thlaspi* spp.) of the Brassicaceae by arbuscular mycorrhizal fungi. J Plant Physiol 160:615–626

Requena N, Breuninger M, Franken P, Ocon A (2003) Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H + -ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiol 132:1540–1549

Rillig MC, Ramsey PW, Morris S, Paul EA (2003) Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. Plant Soil 253:293–299

Rivera-Becerril F, van Tuinen D, Martin-Laurent F, Metwally A, Dietz KJ, Gianinazzi S, Gianinazzi-Pearson V (2005) Molecular changes in *Pisum sativum* L. roots during arbuscular mycorrhiza buffering of cadmium stress. Mycorrhiza 16:51–60

Rizzardo C, Tomasi N, Monte R, Varanini Z, Nocito FF, Cesco S, Pinton R (2012) Cadmium inhibits the induction of high-affinity nitrate uptake in maize (*Zea mays* L.) roots. Planta 236:1701–1712

Saraswat S, Rai JPN (2011) Mechanism of Metal Tolerance and Detoxification in Mycorrhizal Fungi. In: Khan MS, Zaidi A, Goel R, Musarrat J (eds), Biomanagement of Metal-Contaminated Soils, Springer Netherlands, pp 225–240

Scheloske S, Maetz M, Schneider T, Hildebrandt U, Bothe H, Povh B (2004) Element distribution in mycorrhizal and nonmycorrhizal roots of the halophyte *Aster tripolium* determined by proton induced X-ray emission. Protoplasma 223:183–189

Schiavon M, Gallaa G, Wirtz M, Pilon-Smits EH, Telatina V, Quaggiottia S, Hellb R, Barcacciaa G, Malagoli M (2012) Transcriptome profiling of genes differentially modulated by sulfur and chromium identifies potential targets for phytoremediation and reveals a complex S–Cr interplay on sulfate transport regulation in *B. juncea*. J Hazard Mat 239–240:192–205

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Shahabivand S, Aliloo A, Maivan H (2016) Wheat biochemical response to cadmium toxicity under *Funneliformis mosseae* and *Piriformospora indica* symbiosis. Bot Lith 22:169–177

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP (2012) Mycorrhizal networks: mechanisms, ecology and modelling. Fungal Biol Rev 26:39–60

Singh M, Kumar J, Singh S, Singh VP, Prasad SM, Singh MPVVB (2015) Adaptation strategies of plants against heavy metal toxicity: a short review. Biochem Pharmacol 4:161

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Song YY, Ye M, Li C, He X, Zhu-Salzman K, Wang RL, Su YJ, Luo SM, Zeng RS (2014) Hijacking common mycorrhizal networks for herbivore-induced defence signal transfer between tomato plants. Sci Rep 4:3915. https://doi.org/10.1038/srep03915

Spagnoletti F, Lavado RS (2015) The arbuscular mycorrhiza *Rhizophagus intraradices* reduces the negative effects of arsenic on soybean plants. Agronomy 5:188–199

Stommel M, Mann P, Franken P (2001) EST-library construction using spore RNA of the arbuscular mycorrhizal fungus *Gigaspora rosea*. Mycorrhiza 10:281–285

Takahashi R, Ishimaru Y, Nakanishi H, Nishizawa NK (2011) Role of the iron transporter OsNRAMP1 in cadmium uptake and accumulation in rice. Plant Signal Behav 6:1813–1816

Tamayo E, Gómez-Gallego T, Azcón-Aguilar C, Ferrol N (2014) Genome-wide analysis of copper, iron and zinc transporters in the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Front Plant Sci 5:547

Tiberius B, Cătălin T (2011) Interrelations between the mycorrhizal systems and soil organisms. J Plant Dev 18:55–69

Tisserant E, Malbreilb M, Kuoc A, Kohlera A, Symeonidid A, Balestrini R, Charron P, Duensing N, dit Frey NF, Gianinazzi-Pearsoni V, Gilbert LB, Handa Y, Herr JR, Hijri M, Koul R, Kawaguchi M, Krajinski F, Lammers PJ, Masclaux FG, Murat C, Morin E, 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, Tuskans GA, Young JPW, Sanders IR, Henrissat B, Rensing SA, Grigorievc 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 USA 110:20117–20122

Toljander JF, Lindahl BD, Paul LR, Elfstrand M, Finlay RD (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304

Tonin C, Vandenkoornhuyse P, Joner EJ, Straczek J, Leyval C (2001) Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of *Viola calaminaria* and effect of these fungi on heavy metal uptake by clover. Mycorrhiza 10:161–168

Trabelsi D, Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. Biomed Res Int 2013:863240

Trotta A, Varese GC, Gnavi E, Fusconi A, Sampo S, Berta G (1996) Interactions between the soil borne root pathogen *Phytophthora nicotianae* var. parasitica and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209

Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhiza of Berkheya coddii and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. Mycorrhiza 13:185–190

Ultra VU, Tanaka S, Sakurai K, Iwasaki K (2007) Effects of arbuscular mycorrhiza and phosphorus application on arsenic toxicity in sunflower (*Helianthus annuus* L.) and on the transformation of arsenic in the rhizosphere. Plant Soil 290:29–41

Upadhyaya H, Panda SK, Bhattacharjee MK, Dutta S (2010) Role of arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for phytoremediation. J Phytol 2(7):16–27

Vallino M, Massa N, Lumini E, Bianciotto V, Berta G, Bonfante P (2006) Assessment of arbuscular mycorrhizal fungal diversity in roots of *Solidago gigantea* growing in a polluted soil in Northern Italy. Environ Microbiol 8:971–983

Van TN, Ozaki A, Tho HN, Duc AN, Thi YT, Kurosawa K (2016) Arsenic and heavy metal contamination in soils under different land use in an estuary in Northern Vietnam. Int J Environ Res Publ Health 13:1091

Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opin Plant Biol 12:364–372

Viehweger K (2014) How plants cope with heavy metals. Bot Stud 55:1–12

Vodnik D, Grčman H, Maček I, van Elteren JT, Kovačevič M (2008) The contribution of glomalin-related soil protein to Pb and Zn sequestration in polluted soil. Sci Tot Environ 392:130–136

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

Wamberg C, Christensen S, Jakobsen I, Muller AK, Sørensen SJ (2003) The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biol Biochem 35:1349–1357

Warner A, Mosse B (1980) Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. Trans Br Mycol Soc 74:407–446

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53(3):197–201

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

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

Wu T, Ayres E, Li G, Bardgett RD, Wall DH, Garey JR (2009) Molecular profiling of soil animal diversity in natural ecosystems: incongruence of molecular and morphological results. Soil Biol Biochem 41:849–857

Wu Q, Zou Y, Abd-Allah EF (2014) Mycorrhizal association and ROS in plants. In: Ahmad P (ed) Oxidative damage to plants. Academic, Cambridge, MA, pp 453–475

Wu D, Yamaji N, Yamane M, Kashino M, Sato K, Ma JF (2016) The HvNramp5 transporter mediates uptake of cadmium and manganese, but not iron. Plant Physiol 172:1899–1910

Zarei M, Saleh-Rastin N, Jouzani GS, Savaghebi G, Buscot F (2008) Arbuscular mycorrhizal abundance in contaminated soils around a zinc and lead deposit. Eur J Soil Biol 44:381–391

Zhang XH, Lin AJ, Gao YL, Reid RJ, Wong MH, Zhu YG (2009) Arbuscular mycorrhizal colonisation increases copper binding capacity of root cell walls of *Oryza sativa* L. and reduces copper uptake. Soil Biol Biochem 41:930–935

Chapter 5
Microbial Socialization Highlights the AMF Effect

Teresa Dias, Cristina Cruz, Ajit Varma, Juliana Melo, Patrícia Correia, and Luís Carvalho

Abstract Arbuscular mycorrhizal fungi (AMF) are recommended as biofertilizers for sustainable agriculture. So far, most researchers have investigated the effects of AMF on plant growth under highly controlled conditions with sterilized soil. However, it is still poorly documented how the biotic context alone shapes AMF's impact on host plant performance. We inoculated maize (*Zea mays* ssp. *mays*) seedlings with five commercial inoculants of arbuscular mycorrhizal fungi (AMF—*Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora* sp, *Rhizophagus irregularis* and *Scutellospora* sp.). Plants were pot-cultivated for 9 weeks using soil which had been used for maize monocropping in the field. Since we wanted to focus on the impact of belowground interactions (plant-AMF-soil microbes) alone, we compared sterilized versus non-sterilized soil. AMF inoculation was successful, despite an abundant native AMF communities. As hypothesized: (i) the soil biotic context controlled AMF's benefits on maize growth; (ii) AMF's benefits depend on the isolate identity; and (iii) *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overruled soil legacy effects of maize monocropping. We found little to no effects of AMF inoculation on maize growth and nutrients acquisition when plants were grown in sterilized soil. AMFs benefits to their host plants could not be explained by improved nutrition alone because interaction with the remainder soil microbes also differed between inoculated AMF. The results demonstrate that the soil biotic context and AMF isolate identity should be taken into consideration when applying AMF inoculants in agriculture.

T. Dias • C. Cruz (✉) • J. Melo • P. Correia • L. Carvalho
Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisboa, Portugal
e-mail: cruz@fc.ul.pt

A. Varma
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 Block, Fourth Floor, Sector 125, Noida, Uttar Pradesh 201313, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_5

5.1 Introduction

The ongoing human population growth and changing consumption patterns affect food demand and quality, livestock and fibre production, energy use (fossil- and bio-fuel), and land use management (Rockström et al. 2009). As a result, food demand is forecasted to double by 2050 but the environmental footprint must be reduced (for the EU, see Directive 2009/128/EC). This creates an urgent need for cleaner agronomic practices capable of boosting crop yields while alleviating environmental impacts (Dias et al. 2015).

Monocropping is responsible for significant crop yield losses via negative plant-soil feedbacks (or feedbacks). Feedbacks occur because plants 'culture' their interacting soil microbes, which may affect their own growth (e.g. seed germination, seedling survival, individual growth, vegetative propagation and seed production- Bonanomi et al. 2005) and demography as well as that of other plant species (Bever et al. 1997; Bever 2003; van der Putten et al. 2013). Feedbacks can be positive or negative (Bever et al. 1997; Bever 2003). Since increased nutrient availability and plant density shift plant-microbe interactions from mutualistic to neutral or parasitic (Anacker et al. 2014), negative feedbacks in agriculture are well-known since ancient times (Dias et al. 2015). Consequently, so is manipulating plant-microbe interactions in agriculture, namely through crop rotations. Still nowadays, manipulating biotic interactions (e.g. plant-animal, plant-microbe, microbe-microbe) to provide the desired services and thus reduce or eliminate the need for external inputs is fundamental to a cleaner agricultural production. The challenge is to favor positive interactions, while reducing the negative ones (Shennan 2008).

In line with this perspective, there is a steadily growing appreciation of the vital role of soil life in agricultural sustainability (Bender et al. 2016), including plant symbiotic associations. One approach is the use of biofertilizers (i.e. a product containing soil microbes applied to plants to promote their growth- Herrmann and Lesueur, 2013). Among these products, those based on mycorrhizae (the widespread symbioses between fungi and plant roots- Smith and Read 2008) are of special interest because mycorrhizae commonly overrule negative feedbacks on plant growth (Fitzsimons and Miller 2010). Almost all important crops (e.g. maize, wheat, soybean) form associations with arbuscular mycorrhizal fungi (AMF), which are therefore an intricate component of the agrosystem. Examples of AMF's role in agrosystems include pathogen suppression, pollination enhancement, herbivore protection and improved water relations (Verbruggen and Kiers 2010). Despite its enormous potential, the application of AMF has not been fully adopted by farmers so far (Berruti et al. 2016).

AMF generally form mutualisms with plants by trading soil resources and other benefits (e.g. protection from pathogens and stress factors), for photosynthates (Smith and Read 2008). But not all AMF partnerships are equally beneficial for plants; neutral and parasitic AMF symbioses also occur (Johnson et al. 2008). Furthermore, since AMF are obligate biotrophs (Smith and Read 2008), AMF are

often applied in experiments (pot and field trials) and agricultural practices without having in consideration the specificity of the AMF inoculants, compatibility with the target environment and competition with other soil organisms (Berruti et al. 2016). In fact, inoculant production is much more determined by the easiness of growing one isolate than by its effects on plant performance (above a certain positive impact).

Not much is known on how the biotic and abiotic contexts shape biotic interactions, and affect feedback magnitude and direction (Agrawal et al. 2007). AMF are a good model for studying how contextual frameworks affect symbioses, because both biotic and abiotic contexts influence how AMF impact host plant performance (Hoeksema et al. 2010). Given the increasing evidence that non-mycorrhizal soil microbes significantly impact the formation and outcome of the mycorrhizal symbiosis (Frey-Klett et al. 2007), we focused on how the biotic context alone shapes AMF's impact on host plant performance. We chose *Zea mays* subsp. *mays* L. because it is: (i) a fast-growing crop with great economic and nutritional importance worldwide (Ranum et al. 2014); (ii) significantly affected by negative feedbacks (e.g. in the early 1980s, maize monocropping reduced production by 10–15%—http://corn.agronomy.wisc.edu/AA/A014.aspx); and (iii) highly dependent on AMF (Aquino et al. 2015). Since maize is a fast-growing and highly nutrient-demanding crop, we hypothesize that:

1. Subjecting maize to soil legacy effects of maize monocropping will result in negative feedback on plant biomass and nutrients acquisition;
2. Inoculation with AMF will overrule soil legacy effects of maize monocropping.

Negative feedbacks can, non-exclusively, be due to: release of allelopathic compounds by organic matter decomposition (Bonanomi et al. 2005; van de Voorde et al. 2012), nutrient depletion (Bonanomi et al. 2005) and changes in soil microbial communities (including accumulation of pathogens and parasites) (Bever et al. 1997). Since we wanted to focus on the impact of belowground interactions (plant-AMF-soil microbes) alone, from the several feedback approaches (Brinkman et al. 2010; van der Putten et al. 2013), we compared sterilized versus non-sterilized soil. Although decomposition of maize straw releases compounds that may enhance or reduce pathogenicity (Javaid 2008) and affects the following crop (Qi et al. 2015), as far as we know, maize is not auto-allelopathic. To exclude nutrient depletion we used a very poor soil, and to overcome autoclaved-induced increases in nutrients availability (Berns et al. 2008), plants were supplemented weekly with readily available nutrients (Brinkman et al. 2010). Therefore, differences in plant growth between the sterilized and non-sterilized soil treatments will describe the feedback, while differences between AMF isolate treatments will describe interactions of each AMF with the soil microbes (Frey-Klett et al. 2007).

5.2 Experimental Protocol

5.2.1 Experimental Design

Our experimental design consisted of two factors: AMFs inoculation and soil sterilization. The design was fully factorial resulting in 12 treatments with 6 replicates (pots) each. To test if the nutritional benefit to their host plant (symbiont quality) varied between AMF species, we assessed plant response to five AMF species: *Claroideoglomus claroideum*, *Funneliformis mosseae*, *Gigaspora* sp., *Rhizophagus irregularis* and *Scutellospora* sp. To test if symbiont quality was soil biotic community context dependent, we assessed plant response to the presence/absence of a stable soil microbial community (plant-soil feedback). Using soil collected from a maize field in northern Portugal (Vagos, Aveiro—38°29′N−9°1′W) ensured the pre-training of the soil so that there was no need to include a training phase in our experiment.

The soil, at the sampling time, contained 0.4% organic matter, 2.2% humic susbtances, 0.1% total N, 182 ppm total P and 77 ppm K, and had pH (H_2O) 6.5. Available N was 37 ppm while available P and K were 8 and 40 ppm respectively. Soil was mostly composed of sand (>70%), while clay and sand accounted for <30%. Given that mycorrhization is often negatively affected by high nutrient availability, soil was mixed with sterilized river sand in a 1:4 proportion to dilute soil's nutrients. Both sand and soil (only for the sterilized soil treatment) were autoclaved at 121 °C for 1.1 atm for 60 min. Soil and sand were autoclaved three times in consecutive days and then left untouched for a week.

Maize (*Zea mays* L.) seeds (Syngenta) were put under running tap water to remove the antifungal coating and were then sterilized by being placed in ethanol 70% (v/v) for 1 min, then in sodium hypochlorite 2.5% (v/v) for 10 min, and then washed in sterilized distilled water. After sterilization the seeds were germinated in sterilized (70% alcohol) trays containing autoclaved perlite for 5 days and then transferred to the pots. The maize seedlings were planted in 20-cm diameter, 3 L pots (previously sterilized with 70% alcohol) containing the 1-soil: 4-sand mixture. Inoculation was performed a week after seedling transplant.

For each AMF species, six pots were each seeded with 20 g of AMF inoculum containing ~250 AMF spores; an additional six pots were used as controls.

Plants were watered daily with 100 mL of tap water except on the days when they would be supplied with nutrient solution. All plants were fertilised with 100 mL of a 1⁄4 strength Hoagland's solution (1.5 mM KNO_3; 1 mM $Ca(NO_3)_2$; 0.5 mM $NH_4H_2PO_4$; 0.25 mM $MgSO_4$; 50 μM KCl; 25 μM H_3BO_3; 2 μM $MnSO_4$; 2 μM $ZnSO_4$; 0.5 μM $CuSO_4$; 0.5 μM $(NH_4)_6Mo_7O_{24}$; 20 μM FeNaEDTA) every week, which represented the weekly addition of 5.6 mg N; 1.6 mg P; 6.0 mg K; 4.0 mg Ca; 0.6 mg Mg; 0.8 mg S; 27.5 μg B; 177.5 μg Cl; 3.2 μg Cu; 112 μg Fe; 11 μg Mn; 33.6 μg Mo; and 13.1 μg Zn. Plants were grown for 9 weeks, between

July and September 2012, in a greenhouse under a non-sterile environment, with natural light (~15 h day/9 h night), maximum photosynthetic active radiation between 600 and 1000 μmol m^{-2} s^{-1}, and ambient temperature between 17–40°C. Pots were randomized once a week.

5.2.2 Harvest and Analysis

At harvest, maize plants were separated into roots and shoots, and dried at 60 °C until constant mass. Maize shoots were analyzed for macro (nitrogen—N, phosphorus—P, potassium—K, calcium—Ca, magnesium—Mg and sulphur—S) and micronutrients (boron—B, chromium—Cr, copper—Cu, iron—Fe, manganese—Mn, molybdenum—Mo, nickel—Ni and zinc—Zn). The dried plant material was ground into powder using a ball mill (Retsch MM 2000). N concentrations in the plant material were determined using an elemental analyzer (EuroVector) by combustion—DCT (Rodrigues et al. 2009) while the concentrations of all the other nutrients was determined using Inductively Coupled Plasma—Optical Emission Spectroscopy (ICP-OES—Spectro Ciros CCD, Spectro, Germany). We calculated shoot nutrient contents by combining shoot biomass and the respective concentrations. The natural abundance of ^{13}C and ^{15}N in the maize shoots was determined using mass spectrometry (IRMS, Micromass-GV Instruments, UK) and the expressions: $\delta^{13}C = (R\ sample/R\ standard - 1) \times 1000$, where R is the ratio $^{13}C/^{12}C$, in the sample and in the standard and $\delta^{15}N = (R\ sample/R\ standard - 1) \times 1000$, where R is the ratio $^{15}N/^{14}N$, in the sample and in the standard.

To control for effective mycorrhization of the AMF inocula, we evaluated roots' mycorrhizal colonization on plants grown in the sterilized soil: segments of 1 cm length cut 1–2 cm above the root apices. These root segments were stained (Koske and Gemma 1989), and mycorrhizal colonization was evaluated on quadrilateral plaques in accordance with Giovannetti and Mosse (1980) as presence or absence. Another sample of or root tips was used to characterize the microbial community on the root surface and inside the roots (including endophytes) but only for the plants that were hypothesized to suffer negative feedback (those grown in the non-sterilized soil). For that root tips from each of the six replicates per treatment were collected, bulked together in the same proportion, and stored at −20 °C until analysis. DNA was extracted using the GeneMATRIX Plant & Fungi DNA Purification Kit (EURx, Poland). DNA amplification and molecular identification of microorganisms was carried out by sequencing the PCR amplified 16SrRNA gene sequence for prokaryotes (Case et al. 2007) and LO/LOR for fungi (Delgado unpublished). The operational taxonomic units (OTUs) were identified to at least the phylum level.

5.2.3 Calculations and Statistics

Feedback was calculated according to Kardol et al. (2007) as follows:

$$\text{Feedback} = \frac{(\text{Value non sterilized treatment} - \text{Average value sterilized treatment})}{\text{Average value sterilized treatment}}$$

The effect of soil sterilization on plant biomass and on nutrient contents was tested separately using a two-way ANOVA, with soil and AMF treatments as fixed factors. Then, differences between sterilized and non-sterilized soil were analyzed by Student's *t*-test ($p < 0.05$). The effect of the AMF treatments on feedbacks on plant biomass and on nutrient contents was tested separately using a one-way ANOVA, with treatment as fixed factor. Bonferroni post hoc multiple comparisons tested for differences ($p < 0.05$) in feedbacks on plant biomass and on nutrient contents between treatments. Finally, to identify the microbial groups that most contributed to distinguish the microbial communities inhabiting maize roots of plants grown in the non-sterilized soil we used a PCA. For this analysis, the number of sequences per phylum of one sample per each of the six AMF treatments were pooled (n = 6). Preliminary analyses were performed to ensure there was no violation of the assumptions regarding the tests' application. SPSS software, version 23.0, was used for all tests.

5.3 Salient Observations

Only non-inoculated (control) plants grown in sterilized soil were not mycorrhized; plants from all other treatments (including control plants grown in non-sterilized soil) were mycorrhized (data not shown). Despite molecular analysis of the root segments confirmed the presence of the inoculated AMF, for plants grown in the non-sterilized soil it was not possible to conclude whether mycorrhization was done by the inocula or by native AMF.

Control plants grown in sterilized soil accumulated more root, shoot and total biomass than those grown in non-sterilized soil (Fig. 5.1 and Table 5.1). Inoculation with *Rhizoglomus irregularisradices* or *Scutellospora* sp. did not cancel the negative soil feedback (i.e., negative impact of non-sterilized soil) on biomass, while inoculation with *Claroideoglomus claroideum*, *Funneliformis mosseae* and *Gigaspora* sp. enabled maize plants growing in non-sterilized soil to accumulate as much root, shoot and total biomass as those growing in sterilized soil. Since shoot biomass was highly correlated with total biomass ($r = 0.98$; $p = 0.000$), the impacts of AMF and soil sterilization on plant nutrients were assessed on the shoots. Again, inoculation with *R. irregularisradices* or *Scutellospora* sp did not cancel the negative soil feedback (i.e., negative impact of non-sterilized soil) on nutrients, while inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp

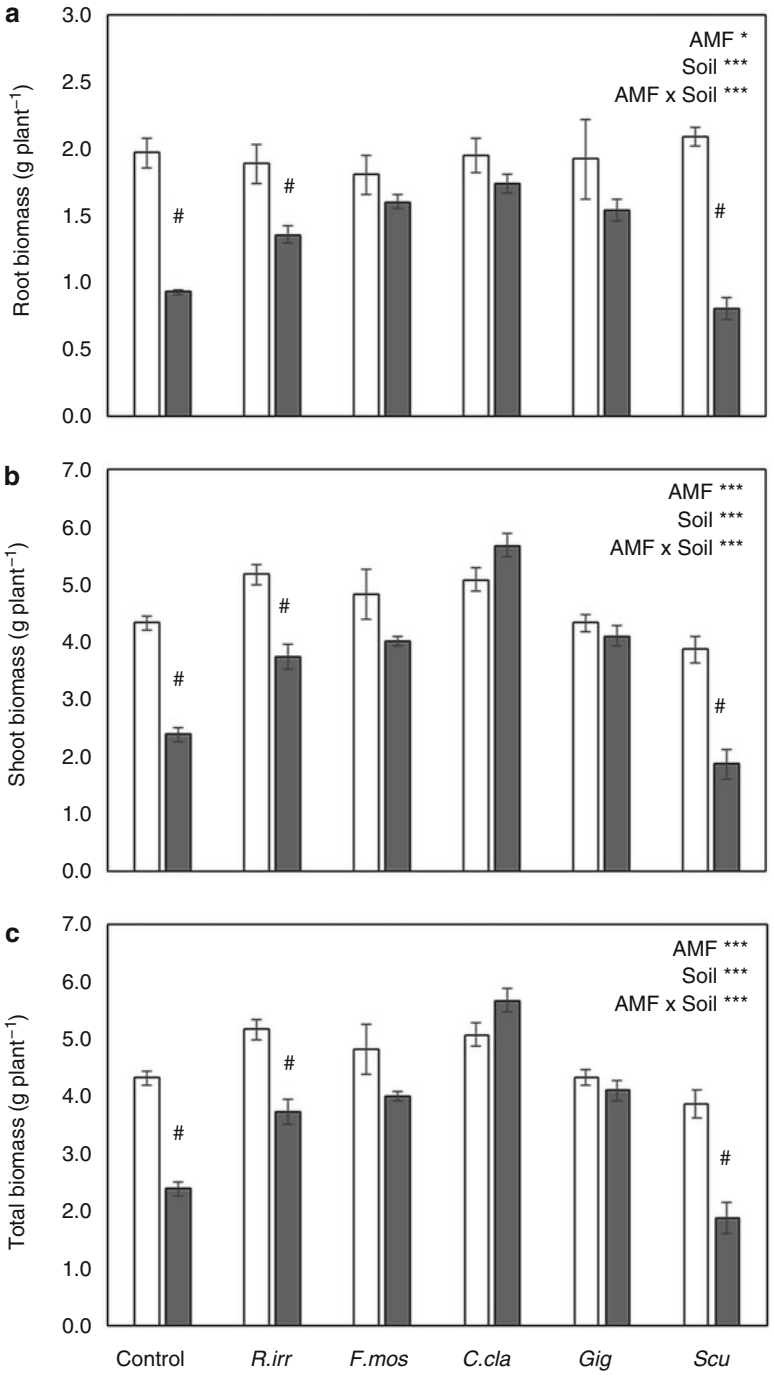


Fig. 5.1 Impact of AMF inoculation and soil sterilization on root (**a**), shoot (**b**) and total plant biomass (**c**). *$p < 0.05$; **$p < 0.01$; ***$p < 0.000$. # shows significant differences between sterilized and non-sterilized soil at the 5% level. Bars are the mean ± 1SE (n = 6)

Table 5.1 Impact of AMF inoculation on soil feedback on plant biomass and on shoot macro- and micronutrients contents

Feedback on:	Control	*C. claroideum*	*F. mosseae*	*Gigaspora* sp.	*R. irregularis*	*Scutellospora* sp.
Root biomass	-0.5 ± 0.0^{c}	-0.1 ± 0.0^{ab}	-0.1 ± 0.0^{a}	-0.1 ± 0.0^{ab}	-0.3 ± 0.0^{b}	-0.7 ± 0.1^{d}
Shoot biomass	-0.5 ± 0.1^{c}	0.1 ± 0.0^{a}	-0.1 ± 0.1^{b}	-0.1 ± 0.0^{ab}	-0.3 ± 0.0^{c}	-0.7 ± 0.1^{d}
Total biomass	-0.5 ± 0.0^{cd}	0.0 ± 0.0^{a}	-0.1 ± 0.1^{ab}	-0.1 ± 0.0^{ab}	-0.3 ± 0.0^{bc}	-0.7 ± 0.1^{d}
Macro**	-0.4 ± 0.0^{bc}	0.0 ± 0.1^{a}	-0.2 ± 0.0^{ab}	-0.1 ± 0.0^{a}	-0.2 ± 0.1^{ab}	-0.5 ± 0.1^{c}
N**	-0.3 ± 0.0^{c}	0.1 ± 0.1^{a}	-0.2 ± 0.0^{c}	0.1 ± 0.1^{ab}	-0.2 ± 0.1^{bc}	-0.4 ± 0.1^{c}
P**	-0.5 ± 0.0^{ab}	-0.1 ± 0.1^{a}	-0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	-0.2 ± 0.1^{ab}	-0.6 ± 0.1^{b}
K**	-0.3 ± 0.0^{ab}	0.0 ± 0.1^{a}	-0.1 ± 0.0^{a}	-0.2 ± 0.1^{a}	-0.2 ± 0.1^{ab}	-0.5 ± 0.1^{b}
Ca**	-0.6 ± 0.0^{bc}	-0.3 ± 0.1^{a}	-0.4 ± 0.0^{ab}	-0.2 ± 0.1^{a}	-0.3 ± 0.1^{a}	-0.7 ± 0.1^{c}
Mg**	-0.5 ± 0.0^{cd}	-0.2 ± 0.1^{ab}	-0.3 ± 0.0^{bc}	0.0 ± 0.1^{a}	-0.3 ± 0.1^{bc}	-0.6 ± 0.1^{d}
S**	-0.4 ± 0.0^{bc}	-0.2 ± 0.0^{ab}	-0.3 ± 0.0^{abc}	-0.1 ± 0.1^{a}	-0.2 ± 0.0^{ab}	-0.6 ± 0.1^{c}
Micro	-0.3 ± 0.1	1.1 ± 1.1	0.5 ± 0.3	-0.5 ± 0.3	-0.8 ± 0.0	-0.3 ± 0.3
B**	-0.6 ± 0.0^{bc}	-0.2 ± 0.0^{a}	-0.3 ± 0.0^{a}	-0.1 ± 0.1^{a}	-0.4 ± 0.1^{ab}	-0.7 ± 0.1^{c}
Cu	-0.8 ± 0.1	-0.7 ± 0.1	-0.6 ± 0.1	-0.7 ± 0.2	-0.5 ± 0.3	-0.8 ± 0.1
Fe	0.0 ± 0.1	2.3 ± 2.0	1.2 ± 0.5	-0.5 ± 0.3	-0.8 ± 0.0	-0.2 ± 0.4
Mn**	-0.8 ± 0.0^{c}	-0.6 ± 0.0^{bc}	-0.5 ± 0.0^{ab}	-0.3 ± 0.1^{a}	-0.7 ± 0.0^{bc}	-0.8 ± 0.1^{c}
Mo	-0.3 ± 0.1	-0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.4	0.2 ± 0.2	-0.4 ± 0.2
Ni	1.0 ± 1.0	5.6 ± 2.9	18.7 ± 12.0	1.4 ± 1.2	0.9 ± 1.3	0.7 ± 0.6
Zn*	-0.6 ± 0.0^{ab}	-0.4 ± 0.1^{ab}	-0.4 ± 0.1^{ab}	-0.4 ± 0.1^{ab}	-0.2 ± 0.2^{a}	-0.7 ± 0.1^{b}

*$p < 0.05$; **$p < 0.01$. Different letters show significance at the 5% level. Values are the mean ± 1SE (n = 6 for biomass and four for nutrients)

enabled maize plants growing in non-sterilized soil to accumulate as much nutrients as those growing in sterilized soil. Therefore, two clusters became evident in terms of AMF's impact on plant biomass and nutrients: (i) inoculation with *R. irregularisradices* and *Scutellospora* sp resulted in a negative feedback, within the same range as that of the control; and (ii) inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overruled the negative soil feedback.

Analysis of roots' microbial community growing in the non-sterilized soil showed that the inoculated AMFs were present in the roots and so were other many other eukaryotes and prokaryotes (data not shown). Principal component analysis (PCA) of the number of sequences of eukaryotes and prokaryotes detected in these roots showed that the first two components explained 76% of the variation (Fig. 5.2). PC1, which explained 35% of the variation, was associated with higher number of bacterial phyla sequences (inoculation with *C. claroideum*, *R. irregularis* and *Scutellospora* sp.), and in the opposite direction, to the number of

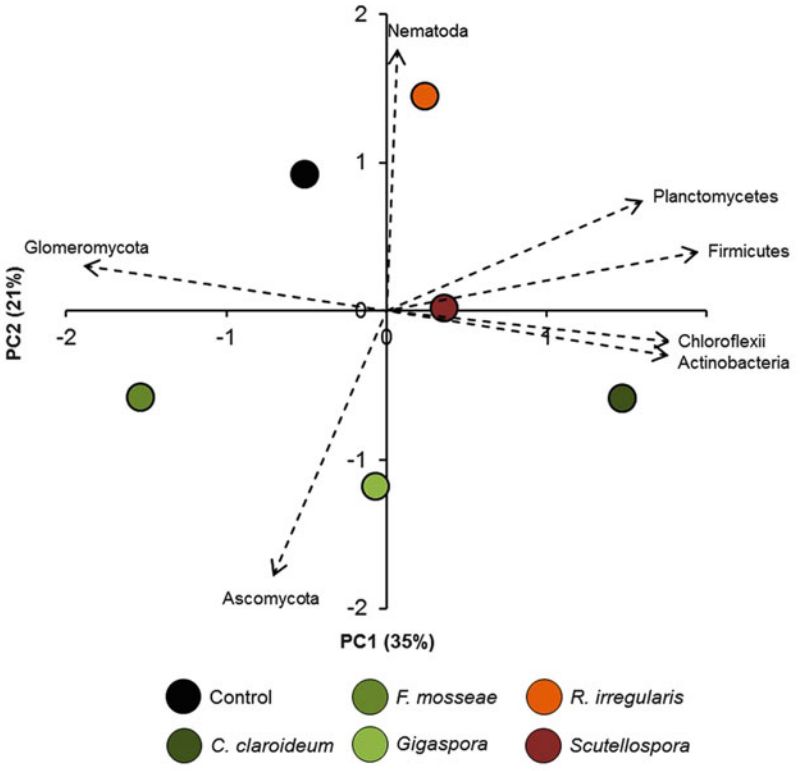


Fig. 5.2 Principal component analysis (PCA) of the root microorganisms (# sequences per phyllum) in the different AMF inoculation in the non-sterilized soil. Symbols represent one bulk sample per treatment; PC1 explains 35% of the variance in the roots microbial community data, PC2 explains 21%. The microbial phylla most responsible for the variations in root microbial community composition (loadings > 0.8) were presented by vectors

Glomeromycota sequences (Control and inoculation with *Gigaspora* sp. and *F. mosseae*). By contrast, PC2, which explained 21% of the variation, grouped the treatments according to the feedbacks on biomass and on nutrients: maize roots from the treatments where plants suffered negative feedback on biomass (Control and inoculated with *R. irregularis* and *Scutellospora* sp.) were associated with higher number of Nematoda sequences, while those where plants did not suffer negative feedback (inoculated with *C. claroideum*, *F. mosseae* and *Gigaspora* sp.) were associated with higher number of Ascomycota sequences. Since PC1 and PC2 contributed in similar ways to explain the variation it is difficult to identify which microbial group(s) would be a particularly strong explanatory gradient influencing roots microbial communities.

5.4 Interpretation of Data

Our study allowed simultaneous examination of plant response to both whole-soil communities and mycorrhizal fractions and showed that: (i) the soil biotic context controls AMF's benefits on maize growth; (ii) AMF's benefits depend on the isolate identity; and (iii) *C. claroideum*, *F. mosseae* and *Gigaspora* sp. overrule soil legacy effects of maize monocropping.

5.4.1 AMF Benefits Depended on the Soil Biotic Context

As expected, the soil legacy effects of maize monocropping resulted in negative feedbacks on plant biomass and nutrient contents (Fig. 5.1 and Table 5.1). The feedbacks on biomass and nutrients we observed resulted from both soil microbes (e.g. bacteria, mycorrhizal or pathogenic fungi) (Kardol et al. 2007) and soil fauna (e.g. nematodes) (Voorde et al. 2012). Both the potential impacts of nutrient depletion (Bonanomi et al. 2005) and of increased nutrient availability due to autoclaving (Berns et al. 2008) were excluded from our study by using a very poor soil and supplying plants with readily available nutrients. Furthermore, the effects of the other growth promoting additives in the tested inocula (e.g. bacteria) were also ruled out from our study by pooling all the additives of each inoculant and adding the same amount of that common extract to each pot (including the controls). So, at time zero the only difference between the treatments was indeed the presence (or absence in the controls) of a certain AMF isolate. Therefore, all the differences observed must be related with the activity of the inoculated AMF: (i) directly on nutrient uptake; and/or (ii) indirectly through distinct interactions with the rhizospheric microbes.

Sterilized and non-sterilized soil differed in soil microbes, including pathogens and parasites (Bever et al. 1997), which interacted differently with the inoculated AMFs. As a result, the plants grown in the sterilized soil grew more than those

grown in the non-sterilized soil, and they also contained more macronutrients (Table 5.1) that are the 'building blocks' of biomass. Surprisingly, and contrary to most studies, we found little to no effects of AMF inoculation on maize growth and nutrients acquisition (Fig. 5.1 and Table 5.1) when the microbes pre-trained by maize monocropping were eliminated by soil sterilization. However, some studies also report a lack of AMF benefits for plants grown in very poor sterilized soils (Ceulemans et al. 2017), likely reflecting severe plant nutrient limitation, together with a lack of 'alternative' nutrient sources to be scavenged by AMF. Non-exclusively, the lack of AMF benefits highlights that mycorrhizal effects can range from fully mutualistic to parasitic interactions, depending on a complex interplay of both partners' identity (Reynolds et al. 2006; Janouskova et al. 2013).

5.4.2 AMF Benefits Depend on the Isolate Identity

Despite an abundant native AMF community that mycorrhized control plants grown in non-sterilized soil, AMF inoculation was successful as shown by the lower biomass and lower nutrient contents in the plants that were not AMF-inoculated (Fig. 5.1 and Table 5.1). These results are in agreement with other studies on AMF inoculation (Vosatka 1995; Kohl et al. 2016). But not all inoculated AMFs conferred benefits to their host plants (van der Heijden et al. 1998; Hart and Reader 2002), which could not be explained by improved nutrition alone because interaction with the remainder soil microbes also differed between inoculated AMF. Due to distinct socialization strategies between inoculated AMFs and the remainder soil microbes and fauna (Fig. 5.2), inoculation with *R. irregularis* and especially with *Scutellospora* sp. did not overrule the soil legacy effects of maize monocropping while inoculation with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. did cancel the negative feedbacks (Fig. 5.1 and Table 5.1).

Maize plants inoculated with *Scutellospora* sp suffered feedback on root and shoot biomass even more negative than that under control conditions (Fig. 5.1 and Table 5.1) thus suggesting mycorrhizal colonization. Since mycorrhized plants experience an initial growth depression compared to non-mycorrhized (Hart and Reader 2002), and *Scutellospora*'s growth is very slow it is possible that *Scutellospora*'s benefits would need longer than the experiment's duration to manifest. This may have implications for the use of this AMF species in crops with short life cycle.

In the absence of the soil legacy effects of maize monocropping (sterilized soil), the plants that presented bigger shoots were those inoculated with *R. irregularis* (Fig. 5.1). However, since plants grew less in the non-sterilized soil than in the sterilized soil, and roots accumulated the most nematodes (Fig. 5.2), inoculation with *R. irregularis* did not overrule the soil legacy effects of maize monocropping (Fig. 5.1). In arable fields, nematode population densities in the upper soil layer can reach 10^7 m^{-2}, the equivalent of 2.0 kg C and 0.25 kg N ha^{-1}. Bacterivores often dominate this fauna, particularly rhabditid and cephalobid species (Bouwman et al.

1996), which were the most abundant nematodes in *R. irregularis* roots. This suggests that nematodes, and possibly other parasites and pathogens decreased *R. irregularis*' efficiency in acquiring nutrients.

By contrast, the roots of plants inoculated with *C. claroideum*, *F. mosseae* and *Gigaspora* sp. accumulated the least nematodes (Fig. 5.2), which is in agreement with other studies (e.g. (Sasanelli et al. 2009; Affokpon et al. 2011). Even though we cannot infer which mechanism(s) caused pathogen protection (changes in root architecture, activation of plant defense mechanisms, competition for infection sites and improved nutrient status, Wehner et al. 2011), the soil legacy effects of maize monocropping was overruled (Fig. 5.1 and Table 5.1). AMFs' role in improving the growth and nutrition of the plant host is widely documented and recognized (Dias et al. 2015) for P (Kothari et al. 1991; van der Heijden et al. 2006, 2008), N (Cruz et al. 2007; Correa et al. 2014, 2015) and micronutrients (Kothari et al. 1991; Liu et al. 2000; Balakrishnan and Subramanian 2012). AMF improve plant nutrition by scavenging 'alternative' nutrient sources that otherwise would not be accessible to plant roots (Smith and Read 1997) and/or by acting as a 'pipeline' of plant-derived C to other soil microorganisms, trading the carbon for nutrients and transferring the nutrients to the plant (Nuccio et al. 2013). Our data does not support the hypothesis that AMF were scavenging 'alternative' nutrient sources since shoot ^{15}N, an integrative indicator of the N source (Ariz et al. 2015), did not change (data not shown). Instead, our data suggest that *C. claroideum*, *F. mosseae* and *Gigaspora* sp. simply extended the root system and thereby took up more nutrients (Smith and Read 1997), and enhanced their host's competitive success against free-living soil microbes (Schimel and Bennett 2004).

5.5 Conclusions

Unlike former observations that AMF are not beneficial in agricultural fields, our results demonstrate that AMF inoculation in field soils can enhance growth of maize irrespective of the pre-established microbial community, being able to compete successfully with indigenous AMF (Kohl et al. 2016). We confirmed clear biological consequences of belowground socialization of AMF with remainder soil microbial communities (biotic context) on plant growth. Furthermore, this effect was AMF species-dependent under a more-structured and stable soil microbial community (i.e., non-sterilized soil) but not under a recently assembled soil microbial community (i.e., sterilized soil), where AMF had little to no effect.

Rhizophagus intraradices, *R. irregularis* and *Funneliformis mosseae* are very generalist symbionts that can colonize a large variety of host plants, survive long-term storage, are geographically distributed all over the world, and can be easily and massively propagated, which makes these species suitable for premium inoculum components. However, our data shows that other AMF (*C. claroideum* and *Gigaspora* sp) may be equally or even more beneficial and should be further assessed for their application in agriculture.

Acknowledgments This study was supported by Portuguese funds through Fundação para a Ciência e a Tecnologia through the projects PTDC/AGRO-PRO/115888/2009 and UID/BIA/00329/2013 (2015–2017), and postdoc grant SFRH/BPD/85419/2012 to Teresa Dias. We are grateful to Fritz Oehl for donating the *Scutellospora* sp. spores and to Steve Houghton for help with the manuscript's preparation.

References

Affokpon A, Coyne DL, Lawouin L, Tossou C, Agbede RD, Coosemans J (2011) Effectiveness of native West African arbuscular mycorrhizal fungi in protecting vegetable crops against root-knot nematodes. Biol Fertil Soils 47:207–217

Agrawal AA, Ackerly DD, Adler F, Arnold AE, Caceres C, Doak DF, Post E, Hudson PJ, Maron J, Mooney KA, Power M, Schemske D, Stachowicz J, Strauss S, Turner MG, Werner E (2007) Filling key gaps in population and community ecology. Front Ecol Environ 5:145–152

Anacker BL, Klironomos JN, Maherali H, Reinhart KO, Strauss SY (2014) Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. Ecol Lett 17:1613–1621

Aquino SD, Scabora MH, Andrade JAD, da Costa SMG, Maltoni KL, Cassiolato AMR (2015) Mycorrhizal colonization and diversity and corn genotype yield in soils of the Cerrado region, Brazil. Semina Cienc Agrar 36:4107–4117

Ariz I, Cruz C, Neves T, Irigoyen JJ, García C, Nogués S, Aparicio-Tejo PM, Aranjuelo I (2015) Leaf δ15N as a physiological indicator of the responsiveness of N2-fixing alfalfa plants to elevated [CO2], temperature and low water availability. Front Plant Sci 6:574

Balakrishnan N, Subramanian KS (2012) Mycorrhizal symbiosis and bioavailability of micronutrients in maize grain. Maydica 57:129–138

Bender SF, Wagg C, van der Heijden MGA (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31:440–452

Berns AE, Philipp H, Narres HD, Burauel P, Vereecken H, Tappe W (2008) Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. Eur J Soil Sci 59:540–550

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. https://doi.org/10.3389/fmicb.2015.01559

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. J Ecol 85:561–573

Bonanomi G, Giannino F, Mazzoleni S (2005) Negative plant-soil feedback and species coexistence. Oikos 111:311–321

Bouwman LA, Hoenderboom GHJ, van der Maas KJ, de Ruiter PC (1996) Effects of nematophagous fungi on numbers and death rates of bacterivorous nematodes in arable soil. J Nematol 28:26–35

Brinkman EP, Van der Putten WH, Bakker E-J, Verhoeven KJF (2010) Plant-soil feedback: experimental approaches, statistical analyses and ecological interpretations. J Ecol 98:1063–1073

Case RJ, Boucher Y, Dahllof I, Holmstrom C, Doolittle WF, Kjelleberg S (2007) Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. Appl Environ Microbiol 73:278–288

Ceulemans T, Bode S, Bollyn J, Harpole S, Coorevits K, Peeters G, Van Acker K, Smolders E, Boeckx P, Honnay O (2017) Phosphorus resource partitioning shapes phosphorus acquisition and plant species abundance in grasslands. Nature Plants 3:16224–16224

Correa A, Cruz C, Perez-Tienda J, Ferrol N (2014) Shedding light onto nutrient responses of arbuscular mycorrhizal plants: nutrient interactions may lead to unpredicted outcomes of the symbiosis. Plant Sci 221:29–41

Correa A, Cruz C, Ferrol N (2015) Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza 25:499–515

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loucao MA, Jakobsen I (2007) Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhizal fungi. Plant Physiol 144:782–792

Dias T, Dukes A, Antunes PM (2015) Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. J Sci Food Agric 95:447–454

Fitzsimons MS, Miller RM (2010) The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. Am J Bot 97:1937–1943

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Giovannetti M, Mosse B (1980) Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

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

Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97:8859–8873

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407

Janouskova M, Krak K, Wagg C, Storchova H, Caklova P, Vosatka M (2013) Effects of inoculum additions in the presence of a preestablished arbuscular mycorrhizal fungal community. Appl Environ Microbiol 79:6507–6515

Javaid A (2008) Allelopathy in mycorrhizal symbiosis in the Poaceae family. Allelopathy J 21:207–217

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) Plant winners and losers during grassland N-euthrophication differ in biomass allocation and mycorrhizas. Ecology 89:2868–2878

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162

Kohl L, Lukasiewicz CE, van der Heijden MGA (2016) Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. Plant Cell Environ 39:136–146

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA-mycorrhizas. Mycol Res 92:486–505

Kothari SK, Marschner H, Romheld V (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil 131:177–185

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013) An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environ Microbiol 15:1870–1881

Qi YZ, Zhen WC, Li HY (2015) Allelopathy of decomposed maize straw products on three soilborn diseases of wheat and the analysis by GC-MS. J Integr Agric 14:88–97

Ranum P, Pena-Rosas JP, Garcia-Casal MN (2014) Global maize production, utilization, and consumption. Ann N Y Acad Sci 1312:105–112

Reynolds HL, Vogelsang KM, Hartley AE, Bever JD, Schultz PA (2006) Variable responses of old-field perennials to arbuscular mycorrhizal fungi and phosphorus source. Oecologia 147:348–358

Rockström J, Steffen W, Noone K, Persson Å, Chapin FS, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, Nykvist B et al (2009) A safe operating space for humanity. Nature 461:472–475

Rodrigues CI, Maia R, Miranda M, Ribeirinho M, Nogueira JMF, Maguas C (2009) Stable isotope analysis for green coffee bean: a possible method for geographic origin discrimination. J Food Compos Anal 22:463–471

Sasanelli N, Anton A, Takacs T, D'Addabbo T, Biro I, Malov X (2009) Influence of arbuscular mycorrhizal fungi on the nematicidal properties of leaf extracts of *Thymus vulgaris* L. Helminthologia 46:230–240

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Shennan C (2008) Biotic interactions, ecological knowledge and agriculture. Philos Trans R Soc B Biol Sci 363:717–739

Smith S, Read D (1997) Mycorrhizal symbiosis. Academic Press, San Diego, CA

Smith SE, Read D (2008) Mycorrhizal symbiosis. Academic Press, New York, NY

van de Voorde TFJ, Ruijten M, van der Putten WH, Bezemer TM (2012) Can the negative plant-soil feedback of *Jacobaea vulgaris* be explained by autotoxicity? J Basic Appl Ecol 13:533–541

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

van der Heijden MGA, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A, Sanders IR (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytol 172:739–752

van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, Van de Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present and future challenges. J Ecol 101:265–276

Verbruggen E, Kiers ET (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evol Appl 3:547–560

Voorde TFJ, van der Putten WH, Bezemer TM (2012) Soil inoculation method determines the strength of plant-soil interactions. Soil Biol Biochem 55:1–6

Vosatka M (1995) Influence of inoculation with arbuscular mycorrhizal fungi on the growth and mycorrhizal infection of transplanted onion. Agric Ecosyst Environ 53:151–159

Wehner J, Antunes PM, Powell JR, Caruso T, Rillig MC (2011) Indigenous arbuscular mycorrhizal fungal assemblages protect grassland host plants from pathogens. PLoS One 6:e27381

Chapter 6
Arbuscular Mycorrhizal Symbiosis and Nutrient Resource Limitation: Predicting the Linkages and Effectiveness of Partnership

Mohammad Haneef Khan, M.K. Meghvansi, Kamal Prasad, Sazada Siddiqui, and Ajit Varma

Abstract Arbuscular mycorrhizal (AM) symbiosis is entirely dependent on nutrient exchange between plant and arbuscular mycorrhizae. The symbioses are up to their fullest under limited conditions of nutrients. There are several theories given to better understand the biological market for their relationship and linkages with the available nutrient resources. However, it is difficult to predict the behavior of relationship between AM fungi and plant for a specific condition. In this chapter we discuss the linkages between AM fungi and nutrient resource limitation surrounding them along with their biochemical and physiological interaction. This discussion is based on individual nutrient limitation (N and P) and co-limitation (N-P and P-K) conditions for AM symbioses. Additionally, this chapter predicts the causality between these nutrient resource limitation and effectiveness of AM symbioses. Therefore, it is of utmost importance to study AM symbioses keeping every component of ecosystem in the loop and not secluded. This chapter also attempts to synthesize the collective and critical information to improve preparedness for effective ecosystem functioning and manage the most unpredictable agro-ecosystems in terms of productivity, biotic and abiotic environment.

M.H. Khan
Professor Joy Michelle Bergelson Lab, Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA

M.K. Meghvansi (✉)
Biotechnology Division, Defence Research & Development Establishment, Gwalior 474002, Madhya Pradesh, India
e-mail: mk_meghvansi@yahoo.co.in

K. Prasad
Symbiosis Sciences Pvt Ltd., Sector-37, Gurgaon 122001, India

S. Siddiqui
Biology Department, College of Science, King Khalid University, Abha, PO, Box 10255, 61321, Saudi Arabia

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

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_6

6.1 Introduction

Arbuscular mycorrhizal (AM) symbiosis is a widely known mutual relationship for nutrient exchange. Phosphorus (P), potassium (K), and nitrogen (N) are among the enlisted nutrients transferred in the form of ions by mycorrhiza in exchange of carbohydrate (Carbon, C) generated during photosynthesis in plants (Smith and Read 2008; Khan et al. 2015). This nearly omnipresent association is recognized in $>$ 70% land plants on the earth (Smith and Read 2008). Notwithstanding the speculation about their evolution, this symbiotic relationship has improved with times and adapted selves with the local environment. The regional conditions include chiefly of abiotic stresses such as of nutrient limitation, salt, drought, pH, and temperature. Among these abiotic stresses, nutrient limitation such as N, P, K limitation (or their co-limitation) is much pervasive phenomena restricting the development of plant in varied ecosystems (Johnson et al. 2010) throughout the terrestrial biosphere and is expected to increase in the future (Elser et al. 2007; Vitousek et al. 2010; Khan et al. 2015; Wieder et al. 2015). These constraints affect the basic physiological and stoichiometric requirements in plants in order to substantiate their net primary productivity (NPP) and biomass (Cleveland et al. 2013). Sprengel-Liebig law of minimum (Liebig 1843; van der Ploeg et al. 1999) stated that nutrient-limited condition may regulate plant productivity. In congruence with this law, spatio-temporal observation of N or P limitation, or their co-limitation across the ecosystems has been known to us since decades (Vitousek and Howarth 1991; Elser et al. 2007; Schmidt et al. 2016). Similarly, K could be one of the growth limiting factors in specific conditions with acidic soil (Jonsson et al. 2003; Thomas et al. 2003; Khan et al. 2015). However, how much low availability of these nutrients are reported, they are never limited in their entirety in the concerned ecosystems. In fact, the differential terrestrial biological productivity depends on both the availability and content of the nutrients (not exclusive to individual nutrient) in a given time and space. The Walker and Syers (1976) model of ecosystem development postulates the concomitant shift of nutrient limitation from soil to plants with N early and P late in ecosystem development (Newman and Hart 2015). The certitude of this shift became apparent by the findings of long-term substrate age gradient studies (Crews et al. 1995; Wardle et al. 2004; Selmants and Hart 2008). Therefore, the study of individual nutrient-limitation will limit the holistic approach of understanding ecosystem functioning. To correctly predict the ecosystem phenomena pertaining to nutrients, stoichiometric reasoning/relationships were introduced (Sterner and Elser 2002). Ecological stoichiometry accentuates the quantitative relationship of chemical constituents considered under ecological interactions (Johnson 2010). Mycorrhiza and plant interaction are paradigm for resource stoichiometric study in the field of ecology. This symbiotic trade involves multiple nutrients and therefore, more rational approach to study resource stoichiometric relationship in order to better understand ecosystem functioning under nutrient limitation. The trade of plant C with mycorrhizae transported soil organic N, P and K at their limited sites are the pivotal selection pressures for the

evolution of effective plant-mycorrhizal partnership (Johnson 2010). Notwithstanding the vast amount of studies performed on AM structure and function, there is a dearth of information in predicting the causality between AM function and nutrient resource limitation. In view of the above, this chapter emphasizes on how the effectiveness of plant-mycorrhizal interaction get influenced with the nutrient limitation.

6.2 AM Symbiosis under P-limited Soil

6.2.1 Plant C vs *Soil P-limitation*

P in soil is immobile but precipitated (Miransari 2013). Despite its abundance, it is found as inorganic phosphate (Pi) forming insoluble complexes chiefly with aluminum and iron under low pH, and with calcium at high pH environments (Smith and Smith 2011). It has been estimated that soluble form of Pi is 10–15% of the total P present in the soil (Johri et al. 2015). Due to this, approx. 30–40% of the global agricultural land is P limited and therefore, use of fertilizers containing P in form of Pi is usual (Gilbert 2009). In addition, the other form of P such as organic Pi and phytates are dependent on pH of the rhizosphere that modulates as the nearby microbial community changes (Rengel and Marschner 2005; Johri et al. 2015). In such cases, AM fungi help plant absorb P as an indirect uptake (Parniske 2008). Now, the question arises that how do fungal mutual relationship respond to soil P crisis. This usually depends on the amount and availability of P in the soil. With high P availability, the host plant does not effuse energy (in form of photosynthate) for symbiotic relationship which leads to the weak mycorrhizal symbiosis. On the contrary, with low to medium P availability, AM fungi develops efficient symbiotic relationship with its host plant by colonizing profusely and therefore, enhance P uptake by plant (Treseder and Allen 2002; Smith and Read 2008; Smith et al. 2011). Liu et al. (2016) examined the diversity of AM fungi in calcareous field and their colonization in maize roots with different P fertilizer application (10–40 ppm) over a 3-year period. They concluded that varied concentration of P fertilization had no significant influence on AM fungal richness. They also found concentration of P is inversely proportional to root colonization. In congruence to similar observation, earlier reports (Olsson et al. 2006; Nouri et al. 2014; Mensah et al. 2015) documented the infected part of the root length and the corresponding number of arbuscules decreases with increase in soil P level. These finding suggests that soil P has strong correlation with AM fungal symbiosis and therefore, a threshold level of soil P must not exceed to forefend effective mycorrhizal development. However, the dependency of efficient mycorrhizal symbiosis on soil P availability and content is not exclusive, but also on plant's photosynthate. Plant invests C reserve in mycorrhizae association that replenishes plant from nutrient limiting conditions (Johnson et al. 2008). This kind of situation abides the optimal foraging theory

(Pyke 1984) which states that plant allocate their biomass to structures that best transfer the limiting nutrient. Therefore, plant's photosynthate regulates the development of efficient mycorrhizal symbiosis under P-limited soil. This is because plant C is the most important trading currency (i.e., cost) than P (Johnson et al. 2010). Further rationale is plant need nutrients for its basic physiological processes, while AM requires plant photosynthate for their growth and life cycle. This draws all attention towards plant for cautious allocation of its photosynthate (8–20%; Rydlová et al. 2016) during AM symbiosis. Johnson (2010) and Smith et al. (2009) illustrated the cost-benefit model wherein the cost of plant C against P uptake benefit under P-limited soil is warranted for an effective AM symbiosis. Nevertheless, C for P trade balance relies on two major factors: (1) Plant competition for AM-dependent P supply under P-limited condition (Li et al. 2008) and (2) AM competition for photosynthate (Smith and Read 2008). This is called resource competition which states that prediction of genotype cohabitation is based on their resource limitation (Tilman 1982, 1988). The theory applies resource ratios (stoichiometry) to envisage the conclusions of the competitive interactions. Although, under P- limited condition, plant genotypes with efficient AM symbiosis have low R* yet, they have competitive advantage over those with inefficient AM symbioses (Johnson 2010). Contrary to this, despite the availability of C in soil as well as plant leaves, the C for P trade between Plant and AM fungi limitative in nature and hence, weakens the symbiotic relationship between them (Khan et al. 2015). Besides these, under AM colonized and P-limitation, expression of gene such as *miR399* increases in plants and through cascade of signals enhance the expression of PiTs (phosphate transporter genes) which eventually ensures the transfer of P flux via AM fungi (Branscheid et al. 2010; Liu et al. 2010; Smith et al. 2011).

6.3 AM Symbiosis under N-limitation Soil

At the global scale the intensity of plant root colonization by AM fungi strongly relates to certain environmental drivers like warm-season temperature, frost periods and soil C:N ratio, and is highest at sites featuring continental climates with mild summers and a high availability of soil nitrogen (Saia et al. 2014).

Traditionally, it was thought that AM fungi played no role in nitrogen (N) acquisition for their host, despite early evidence to the contrary (Govindarajulu et al. 2005; Corrêa et al. 2014; Hodge and Storer 2015). In particular, it was not clear if AM fungi compete with the host plant for the N coming from the decomposing organic matter (OM), especially when the AM extraradical mycelium (ERM) and plant roots share the same soil volume (Saia et al. 2014). Notwithstanding unequivocal evidence that AM fungi can acquire and transfer substantial amounts of nitrogen (N) to their host plants. Fellbaum et al. (2012), reports were contradictory regarding the net effects of AM symbioses on plant growth in N-deficient soil. Some studies showed that AM fungi increase N uptake and

biomass gain of their plant hosts (Tu et al. 2006) whereas others showed that AM symbioses have no benefit for ameliorating N limitation (Reynolds et al. 2005). More recently, this perception has changed radically, with the demonstration that AMF can acquire N from both inorganic and organic N sources and transfer some of this N to their host plant (Hodge and Storer 2015).

AM symbiosis under N-limitation soil basically operated according to the biological market dynamics, in which interaction are viewed from an economic perspective, and the most beneficial partners in resource exchange are favored (Walder and van der Heijden 2015). The resource exchange in this type of symbiosis is determined by competition for surplus resources, functional diversity and sink strength (Walder and van der Heijden 2015). Recent studies suggested that mycorrhizal fungi produce nitrogen-degrading enzymes, allowing them greater access to organic nitrogen sources than arbuscular mycorrhizal (AM) fungi (Averill et al. 2014). It has also been suggested that higher N contents in mycorrhizal plants are just a consequence of an improved supply with P (Reynolds et al. 2005). Hawkins and George (2001), for example, reported that the hyphal N supply was not sufficient to sustain an adequate N nutrition of a host plant under N limitation and found that NH_4^+ reduced the hyphal length in the soil, but not the number of arbuscules, and assumed that high concentrations of NH_4^+ could also have a direct deleterious effect on the ERM. Despite the fungal preference for NH_4^+, when NH_4^+ was the sole N source for mycorrhizal plants, root and shoot biomass, hyphal length densities and N transport via the hyphae to the plant were lower than after NO^{3-} supply (Hawkins and George 2001).

Many ecosystems in which the nitrogen (N) availability in the soil is low and the supply with N often limits plant growth are dominated by mycorrhizal fungi. These fungi can take up inorganic N sources very efficiently from soils (Finlay et al. 1988; Brandes et al. 1998), but their capability to utilize organic N sources, and to make these sources available for the host plant, is generally seen as an important factor in the N nutrition of ECM plant species (Smith and Read 2008). Many ECM fungi can for example mobilize and utilize amino acids and amides, such as glutamine, glutamate and alanine, which can represent a significant N pool, particularly in acid-organic soils (Smith and Read 2008). Some amino acids can be taken up intact, and can directly be incorporated into assimilation pathways and can thereby also represent a significant carbon pool for ECM fungi (Bücking et al. 2012). Even in strongly N-limited boreal forest, a recent study suggested that EMF sustain rather than alleviate plant N limitation by reducing the fraction of fungal N uptake transferred to trees as soil N availability declines (Näsholm et al. 2013). Conversely, experimental N additions increased the proportion of N transferred to the trees and the N:C exchange ratio between fungi and trees, implying a greater symbiotic benefit for the trees at high than at low soil N availability (Näsholm et al. 2013).

AM fungi was reported to increase both plant growth and N uptake. Moreover, AM fungi also increases soil N mineralization rates and total plant N uptake, suggesting that AM fungi have marked effects on competition between plants and bacteria for the different N sources in soil (Saia et al. 2014). Furthermore, it was

also suggested that fertilization reduces the hyphal abundance as well as the specie richness of AM fungi in soil and plant roots (Shi et al. 2014). In low N systems even small amounts of 'extra' N may confer the plant with a competitive advantage, but it is also likely that competition for N between symbionts occurs (Hodge and Storer 2015). Corrêa et al. (2014) studied that mycorrhizal growth response (MGR) was dependent on AM nutrient uptake effects, namely on the synergy between N and Zn, and not on C expenditure. The supply of C to the fungus was dependent on the plant's nutrient demand, indicated by high shoot C/N or low N %. Paradoxically, symbiotic dinitrogen (N_2) fixers are abundant in nitrogen (N)-rich, phosphorus (P)-poor lowland tropical rain forests. N_2 fixers have an advantage in acquiring soil P by producing more N-rich enzymes (phosphatases) that mineralize organic P than non-N_2 fixers. Phosphatase enzymes and AM fungi enhance the capacity of N_2 fixers to acquire soil P, thus contributing to their high abundance in tropical forests (Nasto et al. 2014) (Fig. 6.1).

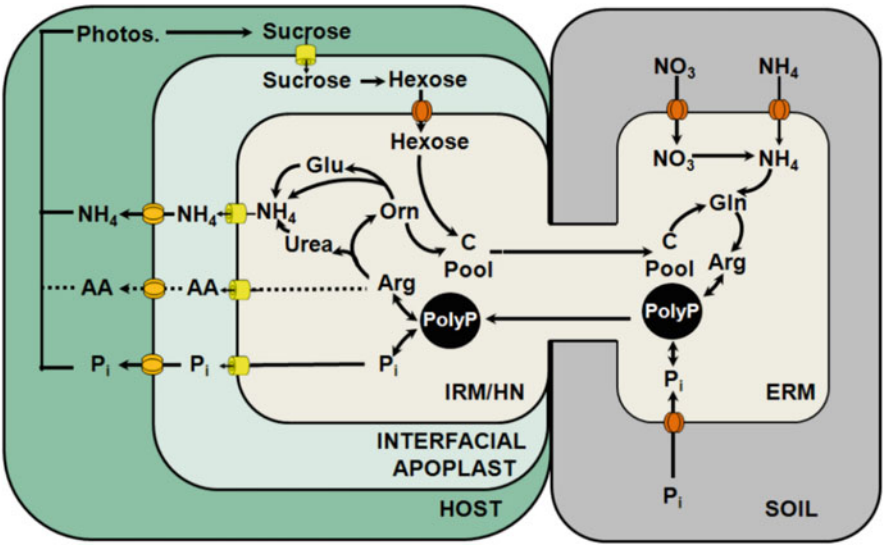


Fig. 6.1 Transport processes in arbuscular and mycorrhizal interactions. The model shows the nutrient uptake by the fungal ERM through Pi, NO_3^- or NH_4^+ transporters (*red*), N assimilation into Arg via the anabolic arm of the urea cycle (only in AM fungi shown) and the conversion of Pi into polyP in the ERM, transport of polyP from the ERM to the IRM, polyP hydrolysis and release of Arg and Pi in the IRM or HN, Arg breakdown to NH_4^+ via the catabolic arm of the urea cycle (only in AM fungi shown), facilitated Pi, NH_4^+ , and potential amino acid (AA, only in ECM postulated) efflux through the fungal plasma membrane (*yellow*) into the interfacial apoplast, plant uptake of nutrients from the mycorrhizal interface through mycorrhiza-inducible Pi or NH_4^+ transporters, stimulation in photosynthesis by improved nutrient supply and facilitated efflux of sucrose through the plant plasma membrane into the interfacial apoplast, sucrose hydrolysis in the interfacial apoplast via an apoplastic plant invertase, and uptake of hexoses by the mycorrhizal fungus through fungal monosaccharide transporters. (Adopted from Bücking et al. 2012)

6.4 AM Symbiosis under N P Co-limitation

Although the plant growth requires more than two dozen elements and the majority of them can be supplied through AMF symbiosis, the most extensively studied relation-ships are those involving the carbon (C): nitrogen (N): phosphorus (P) ratio, because these three elements are tightly interlinked in their biochemical functioning. Nitrogen (N) and phosphorus (P) availability frequently constrain biological processes in terrestrial ecosystems (Vitousek and Howarth 1991; Elser et al. 2007; Vitousek et al. 2010). The contrasting atmospheric- *versus* rock-derived source of N and P, respectively, to ecosystems distinguishes their availability over geologic time and contributes to the differential existence of N or P limitation across temporal and spatial scales. Walker and Syers (1976) model of ecosystem development describes high P availability early in soil development from residual parent materials. Phosphorus availability then declines as P is both eroded from the soil and converted into biologically unavailable forms. Eventually, the ecosystem reaches a 'terminal steady state' of low P availability that is tightly cycled through organic forms. Concurrent with these changes, N is incorporated gradually to the ecosystem via atmospheric deposition and biological N-fixation because N is not present in most parent materials. These differences result in a theoretical shift in soil nutrient limitations to plants from N early in ecosystem development towards a progressive P limitation late in ecosystem development (Walker and Syers 1976; Vitousek et al. 2010). This pattern of shifting N and P availability has been observed in a limited number of established long-term substrate age gradients (Crews et al. 1995; Wardle et al. 2004; Selmants and Hart 2008). Biological responses to these potential nutrient constraints have major implications for the functioning of ecosystems across the landscape and their susceptibility to environmental change. Further, several studies have concluded that multiple resource limitation probably represents the usual situation for terrestrial plants (Bloom et al. 1985; Elser et al. 2007).

Arbuscular mycorrhizal symbiosis is stimulated by P limitation and contributes to P and N acquisition (Smith and Smith 2011). However, the effects of combined N and P limitation on AM formation are largely unknown (Bonneau et al. 2013). The highest AM formation was observed in combined P- and N-limited (LPN), linked to systemic signaling by the plant nutrient status. Plant free phosphate concentrations were higher in LPN as a result of cross-talk between P and N. Transcriptome analyses suggest that LPN induces the activation of NADPH oxidases in roots, concomitant with an altered profile of plant defense genes and a coordinate increase in the expression of genes involved in the methylerythritol phosphate and isoprenoid-derived pathways, including strigolactone synthesis genes (Bonneau et al. 2013).

Although both P and N appear to be important in nutrient transfer during AM symbiosis, little is known about the interconnections between these two elements. Pi is transferred through the mycelium as polyphosphate and released in arbuscules by the action of polyphosphatases (Funamoto et al. 2007). In the extraradical hyphae, N appears to be transported as arginine (Govindarajulu et al. 2005;

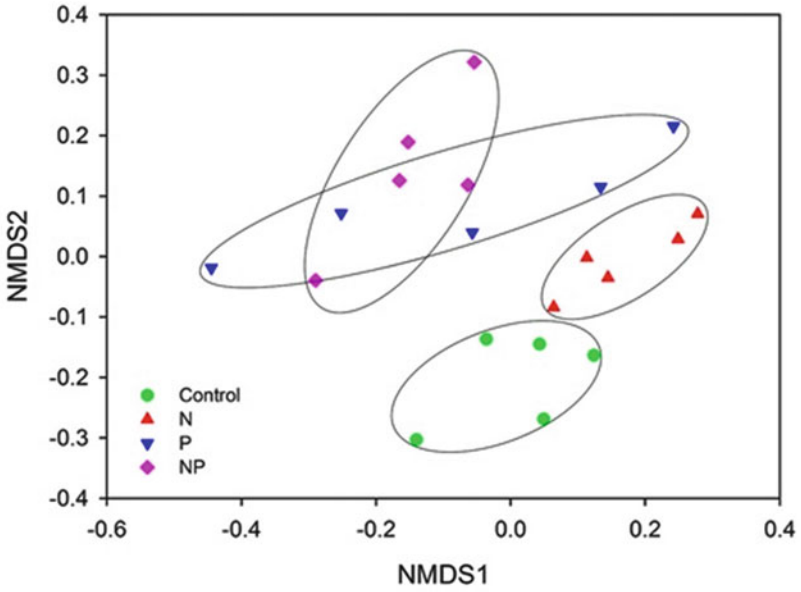


Fig. 6.2 NMDS plot of AMF community composition across control and fertilization (N, P and NP) treatments. (Adopted from Xiang et al. 2016)

Tian et al. 2010) which might bind polyphosphate and therefore be coupled to Pi translocation (Jin et al. 2005). Arginine degradation in the intraradical mycelium releases ammonium which then can be transferred to the host plant (Govindarajulu et al. 2005; Tian et al. 2010).

P- and N-starved plants share common features linked to nutrient deprivation, such as reduced shoot development, and accumulation of starch and anthocyanin. Recently, however, an antagonistic cross-talk between nitrate and Pi concentrations was demonstrated in Arabidopsis, where N-limited plants accumulated more Pi in shoots (Kant et al. 2011) and the *Arabidopsis* NITROGEN LIMITATION ADAPTATION (NLA) gene appears to play an important role in this nitrate-dependent control of Pi homeostasis (Kant et al. 2011) (Fig. 6.2).

Among the mechanisms that could account for the regulation of AM formation, strigolactones, carotenoid-derived signals released by plant roots, were reported to stimulate fungal branching and enhance root colonization in the early stages of symbiosis (Akiyama et al. 2005; Gomez-Roldan et al. 2008). However, as exogenous strigolactones failed to restore root colonization under high-P conditions, other signals remain to be discovered that may contribute to the control of AM formation (Balzergue et al. 2011; Foo et al. 2013). Strigolactones were reported to be produced in response to either P or N deficiency depending on the plant family (Xie et al. 2010). In non-legumes, strigolactone concentrations increased under nitrate deficiency (Yoneyama et al. 2012). It has been speculated that this mechanism would enhance mycorrhizal symbiosis as a contributor of N. However, to date

Table 6.1 Effect of AM fungus colonization on the amount of rhizosphere carboxylates [malate, citrate, lactate, fumarate and oxalate (μmol g^{-1} root DW)] of tobacco plants grown under P-sufficient (Sufficient P, 0.25 mM) or P-limiting conditions (Low P, 0.025 mM)

	Sufficient P		Low P	
	NM	AM	NM	AM
Malate	47 ± 4.2a	24 ± 1.6bc	34 ± 3.2ab	14.5 ± 5.7c
Citrate	14 ± 1.3b	3.6 ± 0.4c	36 ± 2.9a	9.9 ± 4.3bc
Lactate	4.7 ± 1.4	7.9 ± 1.4	16.8 ± 5.8	13.8 ± 6.5
Fumarate	0.62 ± 0.09a	0.54 ± 0.02a	0.13 ± 0.09b	0.15 ± 0.05b
Oxalate	0.71 ± 0.04	0.62 ± 0.06	1.08 ± 0.07	2.6 ± 1.9

Adopted from Del-Saz et al. (2017)

no study has described the impact of combined N and P deficiency on strigolactone synthesis (Bonneau et al. 2013). Also, to date, little attention has been paid to the influence of combined P and N limitation on the establishment and functioning of the symbiosis, and whether the largely unknown mechanisms linking P and N status play pivotal roles in AM formation remains to be elucidated (Table 6.1).

6.5 AM Symbiosis under P K Co-limitation

The functioning of agro-ecosystems is constrained by the harsh environmental conditions, such as low temperatures, acidic soil, and low nutrient supply. P and K limitation are the dominant forms of nutrient limitation in unmanaged terrestrial ecosystems, and are the main controllers of plant growth in the absence of the other growth limiting factors, e.g., light, temperature, diseases and toxicity (Güsewell and Koerselman 2002). In a more recent study on the assessment of elemental stoichiometry and predominant resource limitations influencing the symbioses of four Capsicum genotypes grown on acidic soil at high altitude in Arunachal Pradesh, India the strong evidences of predominant influence of the K-limitation, in addition to P-limitation, on AM symbiosis were observed. These findings necessitated that potassium limitation should also be considered in addition to C, N, and P in further studies investigating the stoichiometric relationships with the AMF symbioses in high altitude agro-ecosystems (Khan et al. 2015). The role of mycorrhizal associations on plant K^+ nutrition has been debated for decades. Although some studies described an improvement of K^+ acquisition in plants colonized by mycorrhizal fungi (Fig. 6.3), others presented apparently contradictory results (reviewed in Garcia and Zimmermann, 2014). The transport of nutrients from the external medium to the host plant through mycorrhizal structures has been investigated using radioactive isotopes (Smith et al. 2011). Because K^+ isotopes are not stable enough to track K^+ movement from the soil to the mycorrhizal plants, some studies have used rubidium as an analog tracer (Hawkes and Casper 2002).

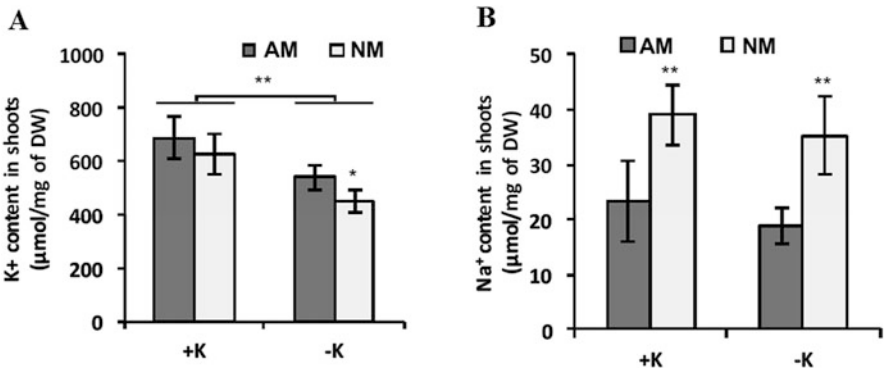


Fig. 6.3 The impact of AM symbiosis on plant fitness under potassium deprivation. Potassium (**a**) and sodium (**b**) content were determined by ICP–OES analysis in shoots of AM and NM plants under $^{+}$K and $^{-}$K conditions. (Adapted from Garcia et al. 2017)

The influence of AM symbiosis on plant mineral nutrition continues to represent a major area of research (Smith and Read 2008) as the outcome is crucial in controlling plant fitness and productivity whilst being relatively easily quantifiable through traditional plant tissue nutrient analysis techniques. Enhanced K assimilation that has been demonstrated in some studies is often linked with low pH. AM fungi appear to positively contribute to plant K nutrition only under acidic conditions (Clark and Zeto 2000). Under P limiting conditions, plant growth is critically dependent on the AM symbiosis in facilitating more efficient P assimilation (Pearson and Jakobsen 1993).

Veresoglou et al. (2011) studied AM species-mediated differences in *P. lanceolate* P and K status manifested in the type of nutrient limitation experienced by the plant host (Fig. 6.4). Large number of studies unequivocally showed that the presence of AMF increases the uptake of K along with the other elements in plants (Cimen et al. 2010). Porras-Soriano et al. (2009) reported that the inoculation of olive plants with AMF increased the plant growth and the plants' abilities to acquire P, and K from non-saline as well as saline media.

6.6 Conclusion

It has been established that the potential of mycorrhizae for agricultural productivity and effective ecosystem functioning is high. Notwithstanding much empirical work has been done on mycorrhizae symbiosis, there is still dire need for obvious understanding of mycorrhizal fungal contributions under limited availability of nutrients especially N and K. To make an effective mycorrhizal symbiosis under nutrient limited conditions, it is imperative to study the causality between native mycorrhizae, plant genotype, and soil properties particularly of nutrients. However, under field conditions plants not only depend on mycorrhizal inoculation, but also

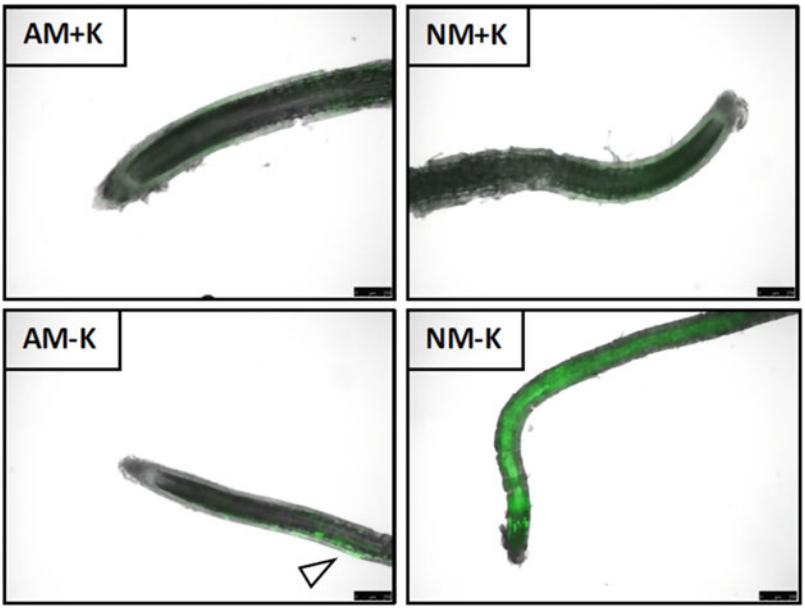


Fig. 6.4 The impact of AM symbiosis and potassium deprivation on the production of reactive oxygen species (ROS) in *M. truncatula*. ROS fluorescence images are shown in AM and NM plants at high ($^{+}$K) or low ($^{-}$K) K^{+} levels. Green pseudocolor indicates ROS production. Bar = 250 μm. (Adopted from Garcia et al. 2017)

on N, P and K supply which has found to be different in varied space and time. This is the rationale why it is pertinent to draw mechanistic causality between arbuscular mycorrhizal symbiosis and nutrient resource limitation. This information may be used to strategize better designing of agricultural and ecosystem management systems. This requires inter-disciplinary approach such as ecosystem modelers, community ecologists and mycorrhizal physiologists, among others to accomplish the task. In addition, to manage indigenous mycorrhizae under long-term field conditions, the effect of soil and nutrient management will be important. Furthermore, promotion of growth of plants in soil, because of mycorrhizal inoculation, may necessarily be associated with characteristics such as N-P-K-uptake, which are manifest under laboratory as well as field conditions and their partnership will prove to be sound.

References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827. https://doi.org/10.1038/nature03608

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505:543–545. https://doi.org/10.1038/nature12901

Balzergue 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. https://doi.org/10.1093/jxb/erq335

Bloom AJ, Chapin FS III, Mooney HA (1985) Resource limitation in plants-an economic analogy. Annu Rev Ecol Syst 16:363–392. https://doi.org/10.1146/annurev.es.16.110185.002051

Bonneau L, Huguet S, Wipf D, Pauly N, Truong H-N (2013) Combined phosphate and nitrogen limitation generates a nutrient stress transcriptome favorable for arbuscular mycorrhizal symbiosis in *Medicago truncatula*. New Phytol 199:188–202. https://doi.org/10.1111/nph.12234

Brandes B, Godbold DL, Kuhn AJ, Jentschke G (1998) Nitrogen and phosphorus acquisition by the mycelium of the ectomycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. New Phytol 140:735–743. https://doi.org/10.1046/j.1469-8137.1998.00313

Branscheid A, Sieh D, Pant BD, May P, Devers EA, Elkrog A, Schauser L, Scheible WR, Krajinski F (2010) Expression pattern suggests a role of MiR399 in the regulation of the cellular response to local Pi increase during arbuscular mycorrhizal symbiosis. Mol Plant Microbe Interact 23:915–926. https://doi.org/10.1094/MPMI-23-7-0915

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. In: Dhal NB (ed) Plant Science. INTECH Open Access Publisher. https://doi.org/10.5772/52570

Cimen I, Pirinc V, Doran I, Turgay B (2010) Effect of soil solarization andarbuscular mycorrhizal fungus (*Glomus intraradices*) on yield and blossom-endrot of tomato. Int J Agric Biol 12:551–555

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902. https://doi.org/10.1080/01904160009382068

Cleveland CC, Houlton BZ, Smith WK, Marklein AR, Reed SC, Parton W, Grosso SJD, Running SW (2013) Patterns of new versus recycled primary production in the terrestrial biosphere. Proc Natl Acad Sci USA 110:12733–12737. https://doi.org/10.1073/pnas.1302768110

Corrêa A, Cruz C, Pérez-Tienda J, Ferrol N (2014) Shedding light onto nutrient responses of arbuscular mycorrhizal plants: nutrient interactions may lead to unpredicted outcomes of the symbiosis. Plant Sci 221:29–41. https://doi.org/10.1016/j.plantsci.2014.01.009

Crews T, Fownes J, Herbert D, Kitayama K, Mueller-Dombois D, Riley R, Scowcroft P, Vitousek PM (1995) Changes in soil phosphorus and ecosystem dynamics across a long soil chronosequence in Hawaii. Ecology 76:1407–1424. https://doi.org/10.2307/1938144

Del-Saz NF, Romero-Munar A, Cawthray GR, Aroca R, Baraza E, Flexas J, Lambers H, Ribas-Carbó M (2017) Arbuscular mycorrhizal fungus colonization in *Nicotiana tabacum* decreases the rate of both carboxylate exudation and root respiration and increases plant growth under phosphorus limitation. Plant Soil 416:1–10. https://doi.org/10.1007/s11104-017-3188-y

Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142. https://doi.org/10.1111/j.1461-0248.2007.01113

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bücking H (2012) Carbon availability triggers fungal nitrogen uptake and transport in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 109:2666–2671. https://doi.org/10.1073/pnas.1118650109

Finlay RD, Ek H, Odham G, Söderström B (1988) Mycelial uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium by *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. New Phytol 110:59–66. https://doi.org/10.1111/j.1469-8137.1988.tb00237

Foo E, Yoneyama K, Hugill CJ, Quittenden LJ, Reid JB (2013) Strigolactones and the regulation of pea symbioses in response to nitrate and phosphate deficiency. Mol Plant 6:76–87. https://doi.org/10.1093/mp/sss115

Funamoto R, Saito K, Oyaizu H, Saito M, Aono T (2007) Simultaneous *in situ* detection of alkaline phosphatase activity and polyphosphate in arbuscules within arbuscular mycorrhizal roots. Funct Plant Biol 34:803–810. https://doi.org/10.1071/FP06326

Garcia K, Zimmermann SD (2014) The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci 5:337. https://doi.org/10.3389/fpls.2014.00337

Garcia K, Chasman D, Roy S, Ane J-M (2017) Physiological responses and gene co-expression network of mycorrhizal roots under K+ deprivation. Plant Physiol. https://doi.org/10.1104/pp.16.01959

Gilbert N (2009) The disappearing nutrient (news feature). Nature 461:716–718. https://doi.org/10.1038/461716a

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot J-P, Letisse F, Matusova R, Danoun S, Portais J-C, Bouwmeeste H, Bécard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455:189–194. https://doi.org/10.1038/nature07271

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823. https://doi.org/10.1038/nature03610

Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations of wetland plants. Perspect Plant Ecol Evol Syst 5:37–61. https://doi.org/10.1078/1433-8319-0000022

Hawkes CV, Casper BB (2002) Lateral root function and root overlap among mycorrhizal and nonmycorrhizal herbs in a Florida shrubland, measured using rubidium as a nutrient analog. Am J Bot 89:1289–1294. https://doi.org/10.3732/ajb.89.8.1289

Hawkins H-J, George E (2001) Reduced ^{15}N-nitrogen transport through arbuscular mycorrhizal hyphae to *Triticum aestivum* L. supplied with ammonium vs. nitrate nutrition. Ann Bot 87:303–311. https://doi.org/10.1006/anbo.2000.1305

Hodge A, Storer K (2015) Arbuscular mycorrhiza and nitrogen: implications for individual plants through to ecosystems. Plant Soil 386:1–19. https://doi.org/10.1007/s11104-014-2162-1

Jin H, Pfefer PE, Douds DD, Piotrowski E, Lammers PJ, Shachar-Hill Y (2005) The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorhizal symbiosis. New Phytol 168:687–696. https://doi.org/10.1111/j.1469-8137.2005.01536

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol 185:631–647. https://doi.org/10.1111/j.1469-8137.2009.03110

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) Plant winners and losers during grassland N-eutrophication differ in biomass allocation and mycorrhizas. Ecology 89:2868–2878. https://doi.org/10.1890/07-1394.1

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci USA 107:2093–2098. https://doi.org/10.1073/pnas.0906710107

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. https://doi.org/10.3389/fmicb.2015.00984

Jonsson U, Rosengren U, Thelin G, Nihlgard B (2003) Acidification-induced chemical changes in coniferous forest soils in southern Sweden 1988–1999. Environ Pollut 123:75–83. https://doi.org/10.1016/S0269-7491(02)00335-4

Kant S, Peng M, Rothstein SJ (2011) Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in *Arabidopsis*. PLoS Genet 7: e1002021. https://doi.org/10.1371/journal.pgen.1002021

Khan MH, Meghvansi MK, Gupta R, Veer V (2015) Elemental stoichiometry indicates predominant influence of potassium and phosphorus limitation on arbuscular mycorrhizal symbiosis in acidic soil at high altitude. J Plant Physiol 189:105–112. https://doi.org/10.1016/j.jplph.2015.10.005

Li H, Smith FA, Dickson S, Holloway RE, Smith SE (2008) Plant growth depressions in arbuscular mycorrhizl symbioses: not just caused by carbon drain? New Phytol 178:852–862. https://doi.org/10.1111/j.1469-8137.2008.02410.x

Liebig J (1843) Chemistry in its application to agriculture and physiology. Taylor and Walton, London

Liu J-Q, Allan DL, Vance CP (2010) Systemic signaling and local sensing of phosphate in common bean: cross-talk between photosynthate and MicroRNA399. Mol Plant 3:428–437. https://doi.org/10.1093/mp/ssq008

Liu W, Zhang Y, Jiang S, Deng Y, Christie P, Murray PJ, Li X, Zhang J (2016) Arbuscular mycorrhizal fungi in soil and roots respond differently to phosphorus inputs in an intensively managed calcareous agricultural soil. Sci Rep 6:24902. https://doi.org/10.1038/srep24902

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 25:533–546. https://doi.org/10.1007/s00572-015-0631-x

Miransari M (2013) Arbuscular mycorrhizal fungi and uptake of nutrients. In: Aroca R (ed) Symbiotic endophytes, Soil biology 37. Springer, Berlin, pp 253–270. https://doi.org/10.1007/978-3-642-39317-4_13

Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN (2013) Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? New Phytol 198:214–221. https://doi.org/10.1111/nph.12139

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC (2014) Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests. Ecol Lett 17:1282–1289. https://doi.org/10.1111/ele.12335

Newman GS, Hart SC (2015) Shifting soil resource limitations and ecosystem retrogression across a three million year semi-arid substrate age gradient. Biogeochemistry 124:177–185. https://doi.org/10.1007/s10533-015-0090-7

Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. PLoS ONE 9:e90841. https://doi.org/10.1371/journal.pone.0090841

Olsson PA, Hansson MC, Burleigh SH (2006) Effect of P availability on temporal dynamics of carbon allocation and *Glomus intraradices* high-affinity P transporter gene induction in arbuscular mycorrhiza. Appl Environ Microbiol 72:4115–4120. https://doi.org/10.1128/AEM.02154-05

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbiosis. Nat Rev Microbiol 6:763–775. https://doi.org/10.1038/nrmicro1987

Pearson JN, Jakobsen I (1993) Symbiotic exchange of carbon and phosphorus between cucumber and three arbuscular mycorrhizal fungi. New Phytol 124:481–488. https://doi.org/10.1111/j.1469-8137.1993.tb03839

Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, Azcón R (2009) Arbuscular mycorrhizal fungi increased growth, nutrient uptake and toleranceto salinity in olive trees under nursery conditions. J Plant Physiol 166:1350–1359. https://doi.org/10.1016/j.jplph.2009.02.010

Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575. https://doi.org/10.1146/annurev.es.15.110184.002515

Rengel Z, Marschner P (2005) Nutrient availability and management in the rhizosphere of plant genotypes. New Phytol 168:305–312. https://doi.org/10.1111/j.1469-8137.2005.01558.x

Reynolds HL, Hartley AE, Vogelsang KM, Bever JD, Schultz P (2005) Arbuscular mycorrhizal fungi do not enhance nitrogen acquisition and growth of old-field perennials under low nitrogen supply in glasshouse culture. New Phytol 167:869–880. https://doi.org/10.1111/j.1469-8137.2005.0145

Rydlová J, Püschel D, Dostálová M, Janoušková M, Frouz J (2016) Nutrient limitation drives response of *Calamagrostis epigejos* to arbuscular mycorrhiza in primary succession. Mycorrhiza 26:757–767. https://doi.org/10.1007/s00572-016-0712-5

Saia S, Benítez E, García-Garrido J, Settanni L, Amato G, Giambalvo D (2014) The effect of arbuscular mycorrhizal fungi on total plant nitrogen uptake and nitrogen recovery from soil organic material. J Agric Sci 152:370–378. https://doi.org/10.1017/S002185961300004X

Schmidt SK, Porazinska D, Concienne B-L, Darcy JL, King AJ, Nemergut DR (2016) Biogeochemical stoichiometry reveals P and N limitation across the postglacial landscape of Denali National Park, Alaska. Ecosystems 19:1164–1177. https://doi.org/10.1007/s10021-016-9992-z

Selmants PC, Hart SC (2008) Substrate age and tree islands influence carbon and nitrogen dynamics across a retrogressive semiarid chronosequence. Glob Biogeochem Cycles 22: GB1021. https://doi.org/10.1029/2007GB003062

Shi G, Liu Y, Johnson NC, Olsson PA, Mao L, Cheng G, Jiang S, An L, Du G, Feng H (2014) Interactive influence of light intensity and soil fertility on root-associated arbuscular mycorrhizal fungi. Plant Soil 378:173–188. https://doi.org/10.1007/s11104-014-2022-z

Smith SE, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

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. https://doi.org/10.1146/annurev-arplant-042110-103846

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. https://doi.org/10.1111/j.1469-8137.2008.02753.x

Smith SE, Jakobsen I, Gronlund 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. https://doi.org/10.1104/pp.111.174581

Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton, NJ

Thomas HM, Morgan WG, Humphreys MW (2003) Designing grasses with a future-combining the attributes of *Lolium* and *Festuca*. Euphytica 133:19–26. https://doi.org/10.1023/A:1025694819031

Tian C, Kasiborski B, Koul R, Lammers PJ, Bücking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175–1187. https://doi.org/10.1104/pp.110.156430

Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton, NJ

Tilman D (1988) Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, NJ

Treseder K, Allen M (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol 155:507–515. https://doi.org/10.1046/j.1469-8137.2002.00470.x

Tu C, Booker FL, Watson DM, Chen X, Rufty TW, Shi W, Hu S (2006) Mycorrhizal mediation of plant N acquisition and residue decomposition: impact of mineral N inputs. Glob Change Biol 12:793–803. https://doi.org/10.1111/j.1365-2486.2006.01149.x

van der Ploeg RR, Böhm W, Kirkham MB (1999) On the origin of the theory of mineral nutrition of plants and the law of the minimum. Soil Sci Am J 63:1055–1062. https://doi.org/10.2136/sssaj1999.6351055x

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. https://doi.org/10.1007/s11104-010-0619-4

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115. https://doi.org/10.1007/BF00002772

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen, phosphorus interactions. Ecol Appl 20:5–15. https://doi.org/10.1890/08-0127.1

Walder F, van der Heijden MG (2015) Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. Nat Plants 1:15159. https://doi.org/10.1038/npalnts.2015.159

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509–513. https://doi.org/10.1126/science.1098778

Wieder WR, Cleveland CC, Smith WK, Todd-Brown K (2015) Future productivity and carbon storage limited by terrestrial nutrient availability. Nat Geosci 8:441–445. https://doi.org/10.1038/NGEO2413

Xiang X, Gibbons SM, He J-S, Wang C, He D, Li Q, Ni Y, Chu H (2016) Rapid response of arbuscular mycorrhizal fungal communities to short-term fertilization in an alpine grassland on the Qinghai-Tibet Plateau. PeerJ 4:e2226. https://doi.org/10.7717/peerj.2226

Xie X, Yoneyama K, Yoneyama K (2010) The strigolactone story. Ann Rev Phytopathol 48:93–117. https://doi.org/10.1146/annurev-phyto-073009-114453

Yoneyama K, Xie X, Kim HI, Kisugi T, Nomura T, Sekimoto H, Yokota T (2012) How do nitrogen and phosphorus deficiencies affect strigolactone production and exudation? Planta 235:1197–1207. https://doi.org/10.1007/s00425-011-1568-8

Chapter 7
Arbuscular Mycorrhiza Mediated Control of Plant Pathogens

Ishwar Singh and Bhoopander Giri

Abstract The application of pesticides in agriculture significantly reduces crop losses by protecting the plant from several diseases, caused by a variety of attackers including fungi, bacteria, plant-parasite nematodes and insects. However, excessive use of these agrochemicals has become a serious cause of concern in agriculture as these not only pose a potential risk to beneficial soil microbes, which play a pivotal role in maintaining the soil-fertility but also, result in serious implications to human health and environment. Researchers are exploring environment-friendly approaches of plant protection that could minimize the side effects associated with the use of pesticides. The biocontrol is a process by which an undesirable organism is controlled with the help of another organism. Among soil microorganisms, arbuscular mycorrhizal fungi (AMF) have demonstrated a considerable potential to reduce crop damages from infectious organisms, whose applications in agriculture have not yet been adopted to a large extent. In view of the importance of AMF in agriculture, we have described the bioprotective role of AMF against various plant pathogens and the possible mechanisms involved in the biological control of crop diseases.

7.1 Introduction

The world's population is growing rapidly and has been predicted to exceed to even nine billion by the middle of this century (Rodriguez and Sanders 2015). Estimations indicate that the challenge to meet the ever-increasing demands of humankind will put a tremendous pressure on the global agriculture for almost doubling the food production. Unrestricted use of chemical pesticides and fertilizers for controlling plant pathogens and increasing food production has brought the world to the brink of an environmental catastrophe. According to the EU directive, in order to

I. Singh
Department of Botany, Hansraj College, University of Delhi, Delhi 110007, India

B. Giri (✉)
Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi, India
e-mail: bhoopg@yahoo.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_7

safeguard the human being and environmental health, and for the sustainable agriculture, it is of paramount importance to decrease the dependence of agrarian population on the use of agrochemicals for preventing the attack of harmful organisms and increasing crop production (EU Directive 2009/128/EC) (Berruti et al. 2016). Therefore, meeting the goal of forecasted agriculture production, it is imperative to implement alternative eco-friendly technologies, such as biocontrol agents, which is based on the sustainable utilization of natural resources. However, regardless of their massive potential, the use of these organisms has not been fully adopted by the farmers so far.

Amongst various biocontrol agents, arbuscular mycorrhizal fungi (AMF) appear to be most promising due to their well-established capability of increasing crop production by helping plants in combating various biotic and abiotic stresses. AMF belonging to the phylum Glomeromycota, develop mutual beneficial relationships with over 90% of terrestrial plants including agricultural and horticultural crops (Schüßler et al. 2001; Smith and Read 2008; Prasad et al. 2017). AM symbiotic association is believed to develop approximately 460 million years ago and perhaps played a major role in establishing the plants on land. In the plant root cortex, the fungal partner of this intimate association develops highly branched treelike structures termed as arbuscules (from Latin "*arbusculum*", meaning bush or little tree), which are the functional sites of nutrient exchange, and balloon-like structures termed as vesicles, which are the storage organ of reserve food (Balestrini et al. 2015). AMF form two types of mycelium system, the intraradical and the extraradical in the plant root and soil, respectively. The extraradical hyphae emerge out from the root system and acquire nutrients from the soil (Miller et al. 1995). AMF extraradical hyphae explore a large volume of soil by extending several meters (extend beyond 100 m) away from the host plant's roots; however, plant root hairs could extend one to 2 mm into the soil. The extensive extraradical mycelium formed by AMF connects many plant's roots to the hyphal network of the fungi (Miller et al. 1995). Further, the fungal hyphae being much thinner than plant roots penetrate the smaller pores more efficiently (Allen 2011). Mycorrhizal fungi have been found to be one of the most abundant organisms of the rhizosphere and play a vital role in the terrestrial ecosystems as several plant species depend on them for the acquisition of mineral nutrients from the soil (Remy et al. 1994). They establish a physical link between plants and soil, provide the host plant with mineral nutrients and improve plant growth and productivity. In exchange, the heterotrophic AMF receive photosynthate from the host plant for the fungal growth, survival, and reproduction (asexual) (Smith and Read 1997, 2008; Goltapeh et al. 2008). Indeed, AMF protect their host from abiotic and biotic stresses (Evelin et al. 2009; Auge et al. 2014; Cameron et al. 2013; Schouteden et al. 2015; Nath et al. 2016), therefore becoming a growing choice as a biofertilizer and bioprotectant (Ijdo et al. 2011; Berruti et al. 2016).

Many studies have shown that AMF improve plant's resistance that consequently reduce incidence as well as severity of plant diseases caused by a wide range of attackers including viruses, bacteria, nematodes and fungi. Although well-defined direct mechanisms are still unclear, several indirect mechanisms have been

proposed by a number of researchers for increasing bioprotective ability of mycorrhizal plants. In the past decades, studied carried out to understand AMF-plant interactions in response to pathogen attack have proposed different mechanisms responsible for the increased tolerance to biotic stress in mycorrhizal plants (Cameron et al. 2013; Schouteden et al. 2015). The involvement of mycorrhizal fungi in the increased nutrient supply and altered rhizosphere microbial population, plant photosynthetic capacity, anatomy and architecture of the root system, root hydraulic conductivity, water use efficiency and antioxidants production seem to play a critical role in the AMF-mediated alleviation of biotic stresses. In this chapter, we have attempted to discuss the protective role of AMF towards different detrimental organisms belonging to fungi, bacteria, viruses and nematodes along with the possible mechanisms that may be employed in mycorrhizal plants with particular emphasis on the activation of defense mechanisms.

7.2 AMF Protection Against Plant Pathogens

Ample literature pertaining to the bioprotective role of AMF against various plant pathogens is available that has been reviewed time to time by various workers (Azcon-Aguilar and Barea 1996; Borowicz 2001; Akhtar and Siddiqui 2008; Veresoglou and Rillig 2012; Bagyaraj 2014; Pereira et al. 2016). Borowicz (2001) conducted a meta-analysis of data related to interactions between AM symbiosis and plant pathogens published between 1970 and early 1998, so that a general pattern, if possible, might be established. This analysis suggested the negative effect of AMF on the growth of pathogens and the nature of interaction between the two was influenced by the identity of the pathogen. AMF reduced pathogen growth in 50% of studies included in meta-analysis. AMF tended to decrease the harmful effects of fungal pathogens but to exacerbate the harmful effects of nematodes. Wherever, there was a negative effect on the growth of nematodes, the outcome depended upon mode of feeding of the nematode. AMF harmed sedentary endoparasitic nematodes but improved growth of migratory endoparasitic nematodes. In 16% of the total experiments included, both AMF and pathogens suffered reduced growth suggesting reciprocal suppression. Reciprocal suppression was more prominent when the pathogen was a fungus indicating that fungal pathogens in comparison to nematodes were more likely to compete with AMF. Another similar meta-analysis that included the wider data of period between 1978 and 2011 also provided unprecedentedly strong evidence of the ability of AMF to suppress plant pathogens. Additionally, the magnitude of the AM-induced decline in disease severity/nematode suppression ranged from 30 to 42% and 44–57% for fungal and nematode pathogens respectively, irrespective of pathogens' identity or lifestyle suggesting that through AMF, plants possibly receive similar protection from all pathogens rendering AM formulations a potentially broadly effective biocontrol agent. Although there were no differences in AMF effectiveness with respect to the identity of the plant pathogen, the identity of

the AMF isolate had a dramatic effect on the level of pathogen protection. AMF efficiency differences with respect to nematode pathogens were mainly limited to the number of AM isolates present; by contrast, modification of the ability to suppress fungal pathogens could occur even through changing the identity of the Glomeraceae isolate applied. N-fixing plants received more protection from fungal pathogens than non-N-fixing dicotyledons; this was attributed to the more intense AMF colonization in N-fixing plants (Veresoglou and Rillig 2012). Considering the bioprotective potential of AMF against pathogens variable results have been reported and the effectiveness of AMF varies with the identity of pathogen and host, and different abiotic factors. The effect of AMF has been studied against various biotic disease causing agents including fungi, bacteria, viruses and nematodes. Since, AMF colonize the roots of higher plants therefore assuming the localized bioprotection most of the studies undertaken are related to soil-borne pathogens and relatively few studies have been conducted on shoot affecting pathogens. On the basis of published reports, it seems that AMF reduce the incidence of disease occurrence in underground parts of plants but enhance the severity of disease in above ground parts in case of certain pathogens (Dehne 1982). AMF species from the family Glomeraceae are more effective at reducing pathogen abundance in contrast to species belonging to family Gigasporaceae (Maherali and Klironomos 2007; Sikes 2010). Further, for promotion and inhibition of plant growth and growth of pathogens, respectively, AMF are known to act synergistically with other microorganisms dwelling in the rhizosphere, which are often termed as plant growth-promoting microorganisms (PGPMs). Amongst PGPMs, plant growth-promoting rhizobacteria (PGPR) such as species belonging to genera, *Pseudomonas*, *Paenibacillus, Rhizobium*, *Stenotrophomonas*, *Arthrobacter* and *Bacillus* and fungi like *Trichoderma* sp., *Gliocladium* sp. and *Aspergillus niger* have been reported to help AMF in reducing the disease severity (Lioussanne 2010; Bharadwaj et al. 2012; Singh et al. 2013; Singh 2015; Prasad et al. 2017; Naglaa et al. 2016; Pereira et al. 2016; Sharma and Sharma 2016). On the basis of information available about mycorrhizal interactions with different pathogens, some of the general conclusions that can be drawn are: (1) AMF either alone or in combination with other microorganisms can reduce damage caused by different pathogens, soil-borne plant pathogens in particular, (2) the abilities of the AM symbioses to enhance resistance or tolerance in roots vary among different AMF tested, (3) the effectiveness of the protection varies from pathogen to pathogen, and (4) protection is influenced by soil and other environmental conditions (Pozo and Azcon-Aguilar 2007). Thus, it can be expected that these interactions will vary with the host plant and the culture system.

7.2.1 Protection Against Fungal Pathogens

The interactions between AMF and fungal plant pathogens have been studied most extensively and majority of these are related to fungal root pathogens

(Whipps 2004). Consistent positive findings related to mycorrhizal plants and their root pathogens have been reported corroborating bioprotective effect of AMF against such pathogens; however, this consistency is missing against shoot pathogens and a lot of variability has been observed that varies with plant and pathogen involved (Ronsheim 2016). The majority of fungal pathogens that have been studied in relation to AMF belong to genera, *Alternaria*, *Aphanomyces*, *Botrytis*, *Colletotrichum*, *Cylindrocladium*, *Erysiphe*, *Fusarium*, *Gaeumannomyces*, *Macrophomina*, *Oidium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotium* and *Verticillium* (Table 7.1).

7.2.2 Protection Against Bacterial Pathogens

There are relatively very few studies where biocontrol effect of AMF has been reported against various bacterial pathogens and majority of these suggest protective role of AMF (Table 7.2). AMF identity appeared to be playing an important role in providing bioprotection against foliar pathogen, *Pseudomonas syringae* pv. *glycinia* on soybean. Amongst AMF, *Entrophospora infrequens*, *Funneliformis mosseae*, *Claroideoglomus claroideum*, and *Racocetra fulgida*, only *E. infrequens* reduced the colonization of pathogen in host plant (Malik et al. 2016).

7.2.3 Protection Against Viral Pathogens

The impact of the AM symbiosis on infections caused by various viral pathogens is still largely uncertain and majority of studies conducted so far indicate negative impact where mycorrhizal colonization increases both multiplication of viruses and severity of the diseases (Dehne 1982; Miozzi et al. 2011), and AMF mediated protection against viral pathogens appeared to be a rare phenomenon (Maffei et al. 2014) (Table 7.3). Yellow mosaic virus infection in mycorrhizal mungbean had adverse effect on mycorrhizal colonization and reduced spore formation by AMF (Jayaram and Kumar 1995). Focusing on the disease symptoms, some of the viruses such as Tobacco mosaic virus (Jabaji-Hare and Stobbs 1984; Shaul et al. 1999), Potato virus Y (Sipahioglu et al. 2009), Citrus tristeza virus and Citrus leaf rurgose virus (Nemec and Myhre 1984), and Tomato spotted wilt virus (Miozzi et al. 2011) (TSWV) have been demonstrated to enhanced disease severity in the presence of mycorrhizal fungi (Table 7.3).

7.2.4 Protection Against Parasitic Nematodes

Nematodes are a diversified group of both free-living nematodes and parasitic nematodes of plants as well as animals that occur world over in various habitats.

Table 7.1 Effect of AMF on different pathogenic fungi in plants

Plant Pathogenic fungus	AM Fungi	Host-plant	Effect[a]	References
Alternaria triticina	*Glomus mosseae*	Wheat	+	Siddiqui and Singh (2005)
A. solani	*Glomus intraradices*	Tomato	+	Fritz et al. (2006)
Aphanomyces euteiches	*G. intraradices*	Pea	+	Bodker et al. (1998)
Botrytis cinerea	*G. mosseae*	Tomato	+	Fiorilli et al. (2011)
Colletotrichum gloeosporioides	*G. mosseae*	Strawberry	+	Li et al. (2010)
Cylindrocladium spathiphylli	*Glomus proliferum, G. intraradices, Glomus versiforme*	Banana	+	Declerck et al. (2002)
Erysiphe graminis	Unknown AMF	Barley	–	Gernns et al. (2001)
Fusarium oxysporum	*Glomus* sp., *G. intraradices*	Yellow foxtail, flax	+	Sikes (2010), Dugassa et al. (1996)
F. udum	*Gigaspora margarita, G. mosseae*	Pigeonpea	+	Siddiqui and Mahmood (1996)
Gaeumannomyces graminis	*G. mosseae*	Barley	+/–	Castellanos-Morales et al. (2011)
Macrophomina phaseolina	*Glomus fasciculatum, Glomus constrictum, Glomus etunicatum, G. intraradices, G. mosseae, G. margarita, Acaulospora* sp., *Sclerocystis* sp.	Chickpea	+	Siddiqui and Akhtar (2006), Shakoor et al. (2015)
Oidium lini	*G. intraradices*	Flax	–	Dugassa et al. (1996)
Phytophthora capsici	*G. mosseae*	Pepper	+	Ozgonen and Erkilic (2007), Pereira et al. (2016)
P. parasitica	*Glomus fasciculatus, G. mosseae*	Tomato, citrus	+	Pozo et al. (2002), Davis and Menge (1980)
P. fragariae	*G. etunicatum, Glomus monosporum*	Strawberry	+	Norman and Hooker (2000)
P. nicotianae	*G. intraradices*	Tomato	+	Lioussanne et al. (2008)
P. sojae	*G. intraradices*	Soybean	+	Yuanjing et al. (2013)
Pythium ultimum	*G. etunicatum*	Cucumber	+	Rosendahl and Rosendahl (1990)
Rhizoctonia solani	*G. etunicatum, G. intraradices, G. mosseae*	Potato, tomato	+	Yao et al. (2002), Kareem and Hassan (2014)

(continued)

Table 7.1 (continued)

Plant Pathogenic fungus	AM Fungi	Host-plant	Effect[a]	References
Sclerotium cepivorum	*Glomus* sp.	Onion	+	Torres-Barragan et al. (1996)
Sclerotium rolfsii	*G. fasciculatum*	Groundnut	+	Doley and Jite (2013)
Verticillium dahliae	*G. mosseae, G. vesiformae, Scutellospora sinuosa*	Cotton	+	Liu (1995)

[a]+ means reduction in disease severity or reduced growth of the pathogen and − means enhanced disease severity or growth of the pathogen

Table 7.2 Effect of AMF against pathogenic bacteria in plants

Plant Pathogenic bacterium	AMF	Host-plant	Effect[a]	References
Pseudomonas syringae pv. *glycinia*	*Entrophospora infrequens*	Soybean	+	Malik et al. (2016)
Ralstonia solanaceraum	G. *mosseae*, *G. vesiforme, Scutellospora* sp., *G. margarita*	Tomato	+	Zhu and Yao (2004), Tahat et al. (2012)
Xanthomonas campestris pv. *alfalfae*	*G. intraradices*	*Medicago*	+	Liu et al. (2007)
Aster yellows phytoplasma	*G. mosseae*	Periwinkle	−	Kamińska et al. (2010)
Stolbur phytoplasma	*G. mosseae*	Tomato	+	Lingua et al. (2002)
Pear-decline phytoplasma	*G. intraradices*	Pear	+	García-Chapa et al. (2004)

[a]+ means reduction in disease severity or reduced growth of the pathogen and − means enhanced disease severity or growth of the pathogen

The plant-parasitic nematodes (PPN) are mostly root pests of a wide range of important agricultural crops and on the basis of feeding strategy, have been classified into different groups viz., ectoparasites, endoparasites, migratory endoparasites, and sedentary parasites (Perry and Moens 2011). Ectoparasitic nematode such as *Xiphinema index* remains in the rhizosphere during feeding and acquire food from the epidermal or outer cortical cells of the roots with the help of the stylet. Endoparasitic nematodes, in contrast, completely enter the root during feeding. Migratory endoparasitic nematodes (e.g., *Radopholus* spp. and *Pratylenchus* spp.) during feeding inside root migrate inter or intracellularly thus, causing damage to the plant along their migration path (Jones et al. 2013). Sedentary endoparasitic nematodes such as cyst and root-knot nematodes are considered to be the most damaging pests of agricultural crops worldwide (Jones et al. 2013; Bartlem et al. 2014) which become sedentary with the onset of feeding in the vascular cylinder (Gheysen and Mitchum 2011). This last group includes various

Table 7.3 Effect of AMF against plant viruses

Plant virus	AMF	Host-plant	Effect[a]	References
Citrus tristeza virus	*G. etunicatum*	Sour orange	–	Nemec and Myhre (1984)
Citrus leaf rurgose virus	*G. etunicatum*	Duncan grapefruit	–	Nemec and Myhre (1984)
Potato virus Y	*G. intraradices*	Potato	–	Sipahioglu et al. (2009)
Tobacco mosaic virus	*Glomus* sp., *G. intraradices*	Tomato, tobacco	–	Jabaji-Hare and Stobbs (1984), Shaul et al. (1999)
Tomato yellow leaf curl Sardinia virus	*Funneliformis mosseae*	Tomato	+	Maffei et al. (2014)
Tomato spotted wilt virus	*F. mosseae*	Tomato	–	Miozzi et al. (2011)
Yellow mosaic virus	*Gigaspora gilmorei, Acaulospora marrowae*	Mungbean	–	Jayaram and Kumar (1995)

[a]+ means reduction in disease severity or reduced growth of the pathogen and – means enhanced disease severity or growth of the pathogen

species of genus *Meloidogyne* such as *M. incognita* and *M. javanica* which, are notorious for severe damages in tobacco, tomato, sunflower and pepper (Wesemael et al. 2011). The direct damage caused by PPN can be aggravated by secondary infections of the wounded plant tissues by other pathogens, moreover, some PPN, like the migratory ectoparasitic *Xiphinema* spp. can transmit plant viruses (Hao et al. 2012). Losses in crops yields caused by PPN are expected to rise due to climate change and cropping systems intensification (Nicol et al. 2011). Considering the limitations of currently used nematicides there is a need for alternative nematode management strategies; the use of AMF has been proposed as one of the environment-friendly strategies to manage PPN. PPN suppressive ability of AMF is well reported and has been previously reviewed (Pinochet et al. 1996; Hol and Cook 2005). In vitro, greenhouse as well as field experiments have indicated protective efficacy of AMF against PPN in crop plants like banana, coffee and tomato (Vos et al. 2013; Alban et al. 2013; Koffi et al. 2013). The bioprotective potential of AMF has been tested in different crop plants with highly variable results against various root-feeding nematodes belonging mainly to the genera *Heterodera*, *Meloidogyne*, *Nacobbus*, *Pratylenchus, Radopholus, Rotylenchulus, Tylenchulus* and *Xiphinema* (Table 7.4).

7.3 Mechanisms of Mycorrhiza-Mediated Disease Control

Mycorrhizal symbiosis largely influences plant growth and development, and also plays a pivotal role in the biocontrol of plant diseases directly or indirectly (Singh et al. 2000; Azcon-Aguilar et al. 2002; Whipps 2004; Xavier and Boyetchko 2004;

Table 7.4 Effect of AMF against different plant-parasitic nematodes

Plant parasitic nematode	AMF	Host-plant	Effect[a]	References
Heterodera cajani	*Glomus epigaeus, G. fasciculatum, G. margarita*	Cowpea, Pigeon pea	+	Jain and Sethi (1988), Siddiqui and Mahmood (1996)
H. glycines	Mixed AMF	Soybean	+	Tylka et al. (1991)
Meloidogyne arenaria	*G. etunicatum, G. margarita, G. fasciculatum*	Peanut, grapevine	−	Carling et al.(1996), Atilano et al. (1981)
M. hapla	*G. etunicatum*	*Pyrethrum*	+	Waceke et al. (2001)
	G. fasciculatum	Onion	−/No effect	Kotcon et al. (1985), MacGuidwin et al. (1985)
	G. intraradices	Tomato	+	Masadeh et al. (2004)
M. incognita	*Acaulospora laevis*	Tomato	+	Labeena et al. (2002)
	G. margarita	Black pepper, tomato	+	Anandaraj et al. (1990), Labeena et al. (2002)
	Glomus aggregatum	Mint	+	Pandey (2005)
	G. mosseae	Tomato, brinjal, okra, black gram	+	Rao et al. (1998), Sharma and Mishra (2003), Vos et al. (2013), Sankaranarayanan and Sundarababu (2010)
	G. fasciculatum	Tomato, brinjal, ginger, black gram	+	Sankaranarayanan and Sundarababu (1997), Borah and Phukan (2003), Nehra (2004), Kantharaju et al. (2005)
	Glomus coronatum	Tomato	+	Diedhiou et al. (2003)
	Glomus deserticola	Tomato	+	Rao et al. (1997)
	Glomus macrocarpum	Tomato	+	Labeena et al. (2002)
	G. etunicatum	Peach	No effect	Strobel et al. (1982)
	Sclerocystis dussi	Tomato	No effect	Labeena et al. (2002)
	G. intraradices	Cotton, chickpea	+	Siddiqui and Akhtar (2006)
M. javanica	*G. mosseae, G. macrocarpum, Glomus caledonicum*	Banana	+	Elsen et al. (2002)
	G. intraradices	Banana	No effect	Pinochet et al. (1997)
	Glomus manihotis, G. margarita, G. gigantea	Chickpea	+	Diederichs (1987)

(continued)

Table 7.4 (continued)

Plant parasitic nematode	AMF	Host-plant	Effect[a]	References
Nacobbus aberrans	*G. intraradices*	Tomato	+	Marro et al. (2014)
Pratylenchus brachyurus	*G. margarita*	Cotton	No effect	Hussey and Roncadori (1978)
P. coffeae	*G. intraradices*	Banana	+	Elsen et al. (2008)
Radopholus citrophilus	*G. intraradices*	Citrus	+	Smith and Kaplan (1988)
R. similis	*G. intraradices*	Banana	No effect/ +	O'Bannon and Nemec (1979), Elsen et al. (2008)
Rotylenchulus reniformis	*G. fasciculatum*	Tomato	+	Sitaramaiah and Sikora (1982)
Tylenchulus semipenetrans	*G. mosseae*	Citrus	+	O'Bannon et al. (1979)
Xiphinema index	*G. intraradices*	Grapevine	+	Hao et al. (2012)

[a]+ means reduction in disease severity or reduced growth of the pathogen and − means enhanced disease severity or growth of the pathogen

St-Arnaud and Vujanovic 2007). In addition to improved plant nutrition, several other mechanisms such as damage compensation, direct competition for colonization sites or photosynthate, alteration in the root morphology, changes in rhizosphere microbial populations, biochemical changes associated with plant defense mechanisms and the activation of plant defense mechanisms have also been found to be involved in AMF-induced biocontrol of plant pathogens (Whipps 2004; Jung et al. 2012) (Fig. 7.1).

7.3.1 Improved Nutrient Status

Although the soluble nutrients such as N and K can be accessed by nonmycorrhizal plants, the less soluble or immobile nutrients like P are more difficult to be attained by plants. This problem further exaggerates under nutrient deficient conditions. Indeed, the extensive thread-like extraradical hyphae of AMF act as an extension of the plant's root system, extend several meters beyond the depletion zone, explore more soil volume and acquire both macro-and micro nutrients, therefore, improve nutrient status of their host plant. The nutritional aspect of plant-AMF interaction has been studied extensively from both physiological and molecular perspectives indicating that AMF considerably improve the acquisition of less soluble or immobile nutrients, mainly under stress conditions (Smith and Smith 2011). Nevertheless, mycorrhizal plants showed better survival and growth and increased resistance

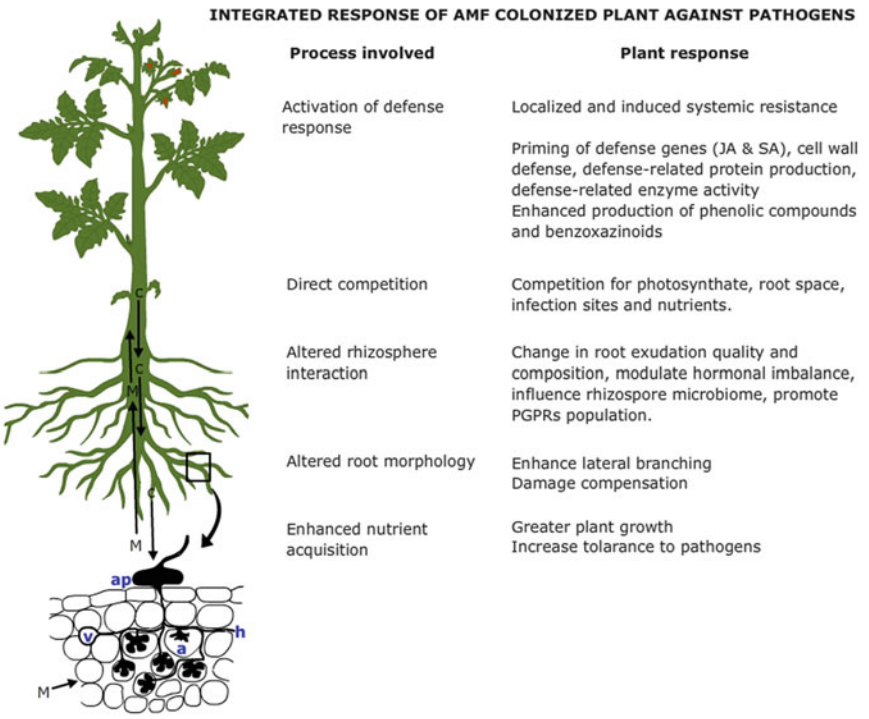


Fig. 7.1 An overview of the processes by which AMF can protect plants from pathogens. AMF facilitate host plant to acquire mineral nutrients (m) from soil and, in exchange, obtain carbon (c) from host plant. AM fungal hyphae enters plant root forming appressorium (ap) on the root surface and eventually vesicles (v) and arbuscules (a) in the root cortical cells by extending its hyphae. The fungus exerts a competition for photosynthate, infection sites and mineral nutrition with infectious organisms. AMF can control plant pathogens by increasing tolerance to pathogens, altering root morphology and rhizosphere interactions, and activating defense responses (ISR, MIR)

or tolerance to plant pathogens than that of the non-AMF plants, which could be ascribed to the increased nutrient status of mycorrhizal plants. However, it remains ambiguous whether the increased resistance against plant pathogens was a direct consequence of improved nutrient acquisition by mycorrhizal plants (Davis 1980; Declerck et al. 2002). Till the date, several reviews have been published advocating improved nutrients supply as one of the main mechanisms of AMF to control plant diseases (Azcon-Aguilar and Barea 1996; Xavier and Boyetchko 2004; Wehner et al. 2010; Schouteden et al. 2015), especially root-borne diseases (Bodker et al. 1998). On the other hand, there are a number of contradictory reports (Hooker et al. 1994; Linderman 1994). Davis (1980) provided an evidence of higher tolerance to citrus root rot disease induced by *Thielaviopsis* in AMF colonized than non-AMF citrus plants, conversely, non-AMF plants exhibited similar response to this pathogen when supplemented with additional dosage of

phosphorus. Declerck et al. (2002) observed higher tolerance to *Cylindrocladium spathiphylli* in mycorrhizal as compared to non-mycorrhizal banana plants. The AMF isolates significantly improved growth and shoot P level of banana, and reduced root damage by *C. spathiphylli*, which may be correlated with the direct interaction between AMF and plant pathogen (Xavier and Boyetchko 2004). Caron et al. (1986) compared response between AMF and non-AMF tomato plants exposed to the *Fusarium oxysporum Schlecht* f. sp. *radicis-lycopersici*, a soil-borne pathogen, which can cause severe yield losses in tomato due to crown and root rot diseases. They observed a reduction in the *Fusarium* population and root rot incidences in the rhizosphere soil of AMF-colonized plants as compared to nonmycorrhizal control plants, and corroborated it with increased antagonistic activities in the mycorrhizosphere, an area influenced by AMF. They suggested that the disease suppression by AMF cannot be considered as a mere consequence of improved P nutrition; instead some other mechanisms may confer reduced disease incidences. A significant suppression in the symptoms of *A. solani* was observed in the mycorrhizal than non-mycorrhizal tomato plants by Fritz et al. (2006). Pettigrew et al. (2005) observed that plants with better nutrient status, growing in the cotton fields, which was infested with the sedentary semi-endoparasitic nematode *Rotylenchulus reniformis* were able to tolerate higher PPN population densities in their roots. Such findings indicate that the improved nutrition of the host plant could influence plant parasite nematode population densities either positively or negatively, however, no solid data are available to confirm a direct correlation between AMF-mediated increased nutrient status of host plant and the higher resistance against plant pathogens.

7.3.2 Changes in the Root System Morphology

The interaction between mycorrhizal fungi and host plant modulates the plant root system and influence microbial activities in the rhizosphere, especially plant-pathogen interaction (Atkinson et al. 1994; Scannerini et al. 2001; Hodge et al. 2009). Consequently, the total root length may increase or remain unchanged as has been reported in *Vitis vinifera* and *Solanum lycopersicum* respectively (Schellenbaum et al. 1991; Berta et al. 2005). The number and length of the roots vary according to the different AMF associations and in comparison to the main roots, modifications occur in the lateral roots more frequently. Pre-inoculation with mycorrhizal fungi increases formation of lateral branching as a result increasing suitable sites for root colonization, which confers to a high branched root system; however, changes in the root architecture as a consequence of AMF colonization could provide a fewer sites for pathogen infection (Norman et al. 1996; Fusconi et al. 1999; Harrison 2005). The strigolactones, a group of sesquiterpene lactones have also been found to play an important role in the modulation of shoot and root architecture. Together with auxins, strigolactones favor lateral root formation and facilitates the root to explore new areas in the soil. Strigolactones-induced changes

in root architecture could alter the dynamics of some pathogen infections; however, further experimentation is required to establish this correlation (Jung et al. 2012).

Mukherjee and Ane (2011) observed that germinating fungal spores of *Gigaspora margarita, G. rosea* and *Glomus intraradices* could induce lateral roots formation and increase the total length of the root system in *M. truncatula*, indicating that diffusible signals released by mycorrhizal fungal spores trigger the lateral root developmental program. The important role of AM symbiosis in lateral root development has also been confirmed by Paszkowski and Boller (2002) and Olah et al. (2005). They revealed that branching in *M. truncatula* is directly induced by AMF germinating spores and their exudates. Increased root branching has also been recorded in the case of monocots and dicots (herbaceous and woody plants) with a significant difference in the order of the roots involved (Berta et al. 1995; Scannerini et al. 2001). However, the tap root plants appear to be more benefited than fibrous root plants in terms of the biomass production and acquirement of mineral nutrients (Yang et al. 2014). The increased root branching in AMF-colonized plants may directly be attributed to the production and action of AMF exudates; while the indirect reason of increasing root branching could be ascribed to the increased mineral nutrition and modulation of hormonal balance (Fusconi 2014). Vos et al. (2014) suggested that increased root branching in AMF-colonized plants could increase the implication for infection by a pathogen; however, a clear correlation did not establish (Vierheilig et al. 2008).

The higher nutrient uptake capacity of mycorrhizal plants attributes to the vigorous root growth, which indeed exhibits higher resistance to plant parasite infection. Elsen et al. (2003a) demonstrated that in banana, migratory endoparasitic nematodes-*Radopholus similis* and *P. coffeae* reduced root branching whereas, same increased by mycorrhizal fungus, *Glomus mosseae*. On the other hand, there is a probability that higher root branching negatively impacts host plant as it increases potential infection sites, depending on the parasite and host plant species. Primary roots are the preferential sites of colonization by migratory endoparasitic nematodes, like *Radopholus similis*, whereas the sedentary endoparasitic root-knot and cyst nematodes prefer to infect the root elongation zone and the sites of lateral root formation (Stoffelen et al. 2000; Wyss 2002; Elsen et al. 2003b; Curtis et al. 2009). Instead, the strawberry plant, bearing highly branched root system, is primarily infected through the root tips by the pathogenic fungus *Phytophthora fragariae*; however, the mycorrhizal roots of strawberry plants showed lower infection as compared to non-mycorrhizal roots, which was indeed higher in the case of highly-branched root system. By the way, it is difficult to explain a uniform impact of altered root morphology in the biocontrol of plant disease, therefore, substantiates the speculations of the involvement of other mechanisms in the mycorrhiza-induced biocontrol of plant diseases (Fusconi et al. 1999; Gamalero et al. 2010).

7.3.3 Changes in the Rhizosphere Interactions

Lorenz Hiltner (1904), a German agronomist and plant physiologist, who coined the term "rhizosphere" and described it as an area around the plant root, which is directly influenced by root secretions and associated population of soil microorganisms (Hiltner 1904). The plant root secretions/exudates comprising of sugars and organic acids (Hage-Ahmed et al. 2013), amino acids (Harrier and Watson 2004), phenolic compounds (McArthur and Knowles 1992), flavonoids (Steinkellner et al. 2007) and plant hormones such as strigolactones, Jasmonic acid, salicylic acid and ethylene (Lopez-Raez et al. 2010; Jung et al. 2012). These compounds play an important role in plant–microbe interactions in the rhizosphere. Some of them are crucial for the initial phase of plant-microbes interactions, while others are required at the later stages of interaction (Bais et al. 2006; Hage-Ahmed et al. 2013).

Alteration in the root exudation patterns directly impacts the microbial population in the mycorrhizosphere. Many studies suggested that AMF alter root exudates quality and quantity (Bansal and Mukerji 1994) and influence the rhizosphere microbial communities, which in turn reduce pathogen populations (Citernesi et al. 1996; Larsen et al. 2003). However, the quality and effectiveness of root exudation indeed depend on the plant and AMF species involved as well as on the degree of root colonization (Scheffknecht et al. 2006; Lioussanne et al. 2008; Badri and Vivanco 2009; Kobra et al. 2009). The findings of Pozo and Azcon-Aguilar (2007) demonstrate the degree of root colonization, which is typically characterized by the presence of arbuscules, as an important requirement for the control of plant pathogen. The root exudates collected from 4 months old AMF-colonized roots was attractive for the zoospores of *Phytophthora nicotianae*, whereas concentrated exudates obtained from 6 months old AMF colonized roots was repulsive for the zoospores of *P. nicotianae*, as compared to exudates harvested from nonmycorrhizal plant roots. This shows that a change in the root exudation pattern in mycorrhizal tomato plants negatively impacts the infection potential of zoospores of *Phytophthora nicotianae*, indicating an obvious involvement of root exudates in the plant-pathogen interactions (Lioussanne et al. 2008). Lioussanne et al. (2008) suggested that modification in the exudates composition of older roots due to AMF colonization could provoke the repulsion of *P. nicotianae*, and therefore reduce the degree of infection. Nevertheless, a differential pattern was also observed in the root exudates of mycorrhizal and non-mycorrhizal plants (Jones et al. 2004; Scheffknecht et al. 2006, 2007). Vos et al. (2013) investigated the influence of a mycorrhizal fungus, *Glomus mosseae and mycorrhizal* root exudates on the *Meloidogyne incognita* penetration of tomato roots. They found that exudates from mycorrhizal tomatoes generally minimize nematode penetration in mycorrhizal plants and transiently inhibited nematodes proliferation as compared to the root exudates obtained from nonmycorrhizal tomatoes. The study showed that nematode penetration of tomato roots decreases in mycorrhizal tomato roots. Moreover, mycorrhizal root exudates could partially affect the motility of

nematode; therefore reduce the *Meloidogyne incognita* penetration and subsequent proliferation in tomato roots. The root exudates obtained from AMF-colonized plants root served as chemoattractants for plant-growth-promoting rhizobacteria, *Azotobacter chroococcum* and *Pseudomonas fluorescens* (Sood 2003). Siasou et al. (2009) found that rhizosphere fluorescent *Pseudomonas* strains produce increasing amounts of antibiotic 2,4-diacetylphloroglucinol in the presence of AMF, *G. intraradices*, which confers plant protection against *Gaeumannomyces graminis* var. *tritici*. This shows that mycorrhizal root exudates positively influence rhizosphere microbial communities and help in the biocontrol of plant pathogens. On the basis of results of many studies, it could be tempting to speculate that mycorrhizal fungi show a protective effect against infectious organisms, which is more obvious in the case of soil-borne diseases, in different plant systems.

7.3.4 Direct Competition with Other Microorganisms

The AMF-host relationship is fundamentally characterized by the development of arbuscules in root cortical cells (Dehne 1982), which die eventually due to their short lifespan. The AMF and pathogenic microbes most probably depend on common resources such as host photosynthate, nutrition, infection-site and space within the plant root for their survival and multiplication (Smith and Read 1997; Whipps 2004); hence competition can occur for these resources between pathogens and mycorrhizal fungi. Indeed, competition can be beneficial in the case of mycorrhiza-colonized cells for the physical exclusion of pathogens (Xavier and Boyetchko 2004). Graham (2001) observed that the competition between plant pathogens and AMF could arise if the availability of carbon is limited, which further corroborates the observations of Vos et al. (2014). The reduced locations for pathogen infection within AMF-colonized root system were also observed by Vigo et al. (2000), which make it plausible that AMF directly compete for colonization sites with other soil microorganisms (Vos et al. 2014). One of the well-studied examples in this connection is the study conducted by Cordier and colleagues on AMF, *Glomus mosseae* and pathogenic fungus, *Phytophthora parasitica* in tomato plants. The study showed that cortical cells of mycorrhizal root did not allow pathogen to penetrate roots that demonstrated to be a localized resistance response (Cordier et al. 1998). Mycorrhizal root system has shown complete exclusion of the *Phytophthora parasitica* from arbusculized cells in tomato roots (Cordier et al. 1996). Therefore, the competition occurring between AMF and pathogens for acquiring host photosynthate, root space or locations could be a potential strategy to control microbial infection in AMF-colonized plants; however, the potentiality of this mechanism to be used as a strategy for control of plant diseases has not received much attention as the results of various studies conducted are not consistent (Pozo et al. 1999; Vos 2012).

Since PPN also colonize plant root, therefore, the competition for space and infection loci appears to be involved in AMF-nematodes interactions. It becomes

very difficult for migratory endo-parasitic nematodes to survive in the presence of mycorrhizal arbuscules, which largely develop in the root cortical cells, where these nematodes feeds upon (Jung et al. 2012; Schouteden et al. 2015). Moreover, the competition for the space between AMF and sedentary endo-parasitic nematodes becomes important if feeding cells extend into the host cortical cells. However, cyst nematodes are meagerly affected by AMF colonization as these nematodes are restricted to the endodermal cells only (Schouteden et al. 2015). Indeed, the formation of mycorrhizal fungi may also be affected by PPN infection as the competition for the nutrients and space arises between them as substantiated by Hol and Cook (2005) who reported reduced AMF colonization due to the attack of migratory and sedentary endoparasitic and ectoparasitic nematodes. Elsen et al. (2003b) observed that mycorrhizal inoculation of *Musa* with *Glomus mosseae* gives rise to a significantly higher plant growth even in the presence of nematodes under greenhouse conditions. Although nematodes decreased the root branching, the population densities of them drastically decreased in the presence of *Glomus mosseae*, and a decrease in root branching seems to be compensated by the AMF inoculation. A negative effect of burrowing nematode *Radopholus similis* and *Pratylenchus coffeae* infection was observed on the frequency of *Glomus mosseae* colonized *Musa* roots (Elsen et al. 2003a, b); however, such effect did not observe in the case of the intensity. In an *in vitro* experiment, carried out by Elsen et al. (2003c), the interaction between AMF and *P. coffeae* on transformed carrot roots in root organ culture did not influence the root colonization by mycorrhizal fungus *Glomus intraradices* (Elsen et al. 2003c), which substantiate the findings of recent study on *in vitro* mycorrhizal banana plantlets and *R. similis* (Koffi et al. 2013). Dos Anjos et al. (2010) observed that the establishment of mycorrhiza prior to the nematode infection could reduce both severity symptoms and reproduction of *Meloidogyne incognita*. Based on the findings, Alban et al. (2013) concluded that pre-inoculation of coffee plants with AMF could develop stronger and healthier plants, which resist *Meloidogyne exigua* infestation and subsequently reduce the rate of infestation. Lower nematode infestation in AMF colonized plants could be attributed to the lignifications of the plant cell wall cuticle, which is not easy to penetrate by nematodes to enter into plant roots. They highlighted that as compared to nematodes, AMF colonize plant root faster, giving them an advantage in their development inside the root, for that reason occupying more root space and eventually lower the nematode infestation rates.

7.4 The Activation of Defense Mechanisms in Response to AMF Formation

For the biological control of plant diseases, it is expected that arbuscular mycorrhizal fungi could apply more than one mechanism to restrain the adverse effects of the causal organisms. As above mentioned mechanisms do not elucidate

satisfactorily the mycorrhiza-induced biocontrol of certain plant diseases, therefore, researchers have explored possibilities of other mechanism like the activation of plant defense compounds in response to AMF formation in plant roots. It has been observed that, in response to mycorrhizal colonization, though a weak or very local and transient, activation of specific plant defense compounds takes place which, elicits the specific defense reactions and makes the plants proactive against attackers/pathogens (Gianinazzi-Pearson et al. 1994; Koide and Schreiner 1992). The chemicals produced under plant defense mechanism are diversified groups of biochemicals which are synthesized mostly as secondary metabolites (Singh 2017). Some of such chemicals like, phytoalexins, chitinases, β-1,3-glucanases, pathogenesis-related proteins, callose, hydroxyproline-rich glycoproteins, phenolic compounds and enzymes of the phenylpropanoid pathway have been reported to be involved in the activation of plant defense as a result of AMF colonization (Gianinazzi-Pearson et al. 1994; Azcon-Aguilar and Barea 1996).

In the past decades, the changing host plant physiology (including plant nutrition) as a consequence of plant-AMF interactions and the plant signal transduction pathways controlling this intimate association have largely been the central point of research. Conversely, a little attention has been paid to understand the contribution of AMF for non-nutritional benefits, such as suppression of plant diseases or induction of plant resistance against plant diseases/attackers. It is well established that plant roots exude different chemical compounds (secondary metabolites), which usually signaling an array of soil microorganisms, predominantly occurring in the rhizosphere. The pioneer observations of Akiyama et al. (2005) and successive publications have established that strigolactones, a group of carotenoids-derived signaling molecules that are released by the host plant root promote AMF-hyphal branching and facilitate AMF establishment in the plant root. The production of strigolactones generally increases in the early phase of root colonization but, it decreases in a well-established mycorrhizal symbiosis (Jung et al. 2012). The root exudates released by AMF-colonized plants seems to prime microbial activities in the rhizosphere and thereby induce a 'mycorrhizosphere effect' (Linderman 1988; Giri et al. 2005).

Like certain other soil microbes, mycorrhizal fungi also exhibit the ability to reduce the impacts of plant pathogens through induced systemic resistance (Pozo and Azcon-Aguilar 2007). The induced systemic resistance (ISR) is typically a sustained induction of resistance in plants against pathogens, which is induced by the non-pathogenic rhizosphere microorganisms such as PGPR, mycorrhizal fungi or endophytes (Trotta et al. 1996; Cordier et al. 1998; Pineda et al. 2010). ISR protect plants from the infection of different plant pathogens including bacteria, fungi, viruses, nematodes and insects and is mediated by the priming of defense genes that demonstrate a higher expression systemically in the leaves only after the pathogen attack (van Wees et al. 2008; Pineda et al. 2010). ISR often exhibits increased plant-sensitivity towards the plant growth regulators such as jasmonic acid (JA) and ethylene (ET) (Pineda et al. 2010).

In the AMF-colonized plants, both localized and induced systemic resistance has been observed against detrimental organisms. Cordier et al. (1998), in split root

experiment observed the protective role of AMF, *Glomus mosseae* against *Phytophthora parasitica* as it hindered the development and proliferation of causal organisms in tomato roots. They suggested that root cortical cells become immunized on AMF colonization resulting in a localized plant cell resistance, likely due to the formation of cell wall appositions supported by callose (adjacent to intercellular hyphae) and phenolic compounds, and plant cell defense responses. Induced systemic resistance in nonmycorrhizal root parts of the mycorrhizal plants could attribute to the host wall-thickenings containing non-esterified pectins and pathogenesis-related (PR)-1a protein and formation of callose rich jacket around the hyphae of *P. parasitica* (Cordier et al. 1998). Liu et al. (1995) found more than ten types of pathogenesis-related (PR) proteins in cotton plants, moreover, the content of PR proteins increased on AMF inoculation. The growth of hyphae and conidia of *Verticillium dahlia* were found to be hampered at certain concentrations in mycorrhizal plants, which subsequently helped in the reduction of disease incidences and index of verticillium wilt and in the promotion of seedling growth and yield of cotton seed. Indeed, the higher MIR response against *V. dahlia* could be an effect of the increased content of PR proteins in cotton plants on mycorrhization.

Mycorrhiza-induced resistance (MIR) against many plant pathogens has been demonstrated by researchers that seems to be based more on the priming of jasmonic acid-dependent defense (JADD) responses (Pozo and Azcon-Aguilar 2007) than accumulation of salicylic acid (Khaosaad et al. 2007) as there are reports suggesting negative impact of salicylic acid on mycorrhizal colonization (Hause et al. 2007; Jung et al. 2012; Miransari and Smith 2014). Priming can be explained as a pre-conditioning of plant tissues that allows the plant to activate defense responses faster and stronger against a broad range of plant pathogens/attackers. Such a pre-conditioning can be elicited by certain chemical compounds and different microorganisms (Pineda et al. 2010). Priming seems to be a plausible mechanism operating in MIR. The MIR-regulated bioprotection of crop plants has been reported in the case of *Gaeumannomyces graminis* var. *tritici*, which causes Take-all disease in barley (Khaosaad et al. 2007). Aberra et al. (1998) also observed similar results in the case of a sterile red fungus, which suppressed the infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Benhamou et al. (1994) observed strong defense reactions at the sites challenged by *Fusarium oxysporum* f. sp. *chrysanthemi* in mycorrhizal transformed carrot roots. The protective ability of mycorrhizal fungi in grapevine roots against *Meloidogyne incognita*, has been found to be associated with primed expression of a chitinase gene (VCH3) (Li et al. 2006). Moreover, mycorrhiza-induced resistance against *Fusarium oxysporum* in mycorrhizal date palm trees and *Colletotrichum* in cucumber seem to be associated with the primed-accumulation of the phenolic compounds (Jaiti et al. 2008), and callose deposition (Lee et al. 2005), respectively.

Fritz et al. (2006) conducted an experiment to understand the influence of mycorrhizal fungi on the development of early blight in tomato, caused by a necrotrophic fungus, *Alternaria solani*. The mycorrhizal tomato plants showed a significant reduction in disease symptoms as compared to the non-mycorrhizal plants. Although the increased nutrient supply considerably influenced disease

severity in mycorrhizal than non-mycorrhizal plants, the defensive role of AMF against causal organisms could be due to mycorrhiza-induced resistance. Recently, Cameron et al. (2013) presented a four-phase spatiotemporal model demonstrating MIR as a cumulative outcome of direct interactions between mycorrhizal fungi and plant, and responses to ISR-eliciting rhizobacteria present in the mycorrhizosphere. They highlighted that at the early stage of colonization, strigolactones present in the exudates of host plant roots, promote hyphal branching and AMF establishment. At the onset of mycorrhizal symbiosis, microbe-associated molecular patterns (MAMPs) from the fungus are recognized by the plant's innate immune system that leads to a weak and transient MAMP-triggered immunity (MTI) response as well as the activation of long-distance signals in the xylem, which could prime the salicylic acid-dependent defense (SADD) responses. The AMF-regulated induction of the specific effector molecules suppresses MTI locally. Mycorrhizal symbiosis elicits the production of abscisic acid that systemically transports to the shoot tissues through xylem and can prime cell wall defense responses. With the development of arbuscules in the root cortical cells, the increased supply of sugar and mineral nutrition, particularly phosphorus modulates root metabolisms and thereby the chemical composition of root exudates. Such modifications in the root exudates chemical composition indeed influence mycorrhizosphere populations, which could allow the selection of growth-promoting rhizosphere microbes/bacteria that metabolize AMF root exudates and convey ISR-eliciting signals at the root surface and/or AMF hyphae, resulting in the long-distance signals, which could credit the priming of JA-dependent defense and ET-dependent defense responses and eventually triggers the ISR in mycorrhizal plants (Cameron et al. 2013). Although the well-defined roles of jasmonic acid in MIR against detrimental organisms are not well known, Pozo and Azcon-Aguilar (2007) proposed that the active suppression of components involved in the SA-dependent defense pathway could lead to the induction of MIR and the priming of JA-dependent defense responses (Pozo and Azcon-Aguilar 2007; Hause et al. 2007). Although salicylic acid has been proved to be a key regulator of plant defense against biotrophs (Glazebrook 2005), it could have negative impact on AMF formation in the host roots (García-Garrido and Ocampo 2002; Lopez-Raez et al. 2010). Therefore, it is possible that AMF suppress certain components of SA-dependent responses in the host plant in order to accomplish a compatible interaction. Dumas-Gaudot et al. (2000) also observed a delay in the accumulation of PR-1 proteins (common markers of SA-dependent responses) in mycorrhizal roots. Nevertheless, the beneficial microbes (PGPR)-induced systemic resistance, regulated by JA-dependent and ethylene-dependent signaling pathways, and associated with priming for enhanced defense against plant pathogens has been observed by van Wees et al. (2008). These findings tempting to consider that the JA is a key regulator in the AMF symbiosis (Hause et al. 2007; Hause and Schaarschmidt 2009). The higher levels of JA have been confirmed in mycorrhizal tomato roots (Lopez-Raez et al. 2010), which remains unchanged in the shoots (Pozo et al. 2010). Pozo et al. (2010) recorded rather low, but a significant increase in the expression of marker genes for JA responses and a strong induction of JA-regulated genes in *Glomus mosseae*-colonized tomato plants.

Confirming a priming response in tomato plant they further validated the MIR response against *Botrytis cinerea* directly related to the priming of JA-dependent defense responses.

7.5 Conclusions and Outlook

Arbuscular mycorrhizal fungi have been found to protect several plant species against many pathogens including viruses, phytoplasmas, bacteria, fungi, and plant-parasite nematodes. The suppression of both necrotrophic and biotrophic pathogens and consequently reduction in the severity of disease symptoms either directly or indirectly has also been observed in majority of AMF-colonized plants. However, soil-borne root attackers appear to be affected more than shoot attackers. One of the reasons of such a generalization is availability of relatively few reports pertaining to air-borne pathogens. The protective action mechanism of AMF is not fully understood and based on the investigations done so far appears to be multi-targeted. The primary protection mechanism is increased resistance of crop plants as a result of AMF-mediated improved nutrient status. However, studies indicate the involvement of other mechanisms such as changes in the chemical composition of root exudates, modulation of rhizosphere microbial activities and the activation of plant defense responses in mycorrhiza-induced protection against different attackers. Indeed, the actual use of AMF as a bio-control agent remains to be popularized for agricultural practices.

Acknowledgements Authors duly acknowledge the support provided by Dr. Bhawna Saxena, Swami Shraddhanand College, University of Delhi, Delhi, India throughout the preparation of Manuscript.

References

Aberra MB, Seah S, Sivasithamparam K (1998) Supression of the takeall fungus (*Gaeumannomyces graminis* var. *tritici*) by a sterile red fungus through induced resistance in wheat (*Triticum aestivum*) seedling roots. Soil Biol Biochem 30:1457–1461

Akhtar MS, Siddiqui MA (2008) Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Siddiqui ZA, Akhtar S, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Netherlands, pp 61–98

Akiyama K, Ki M, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Alban R, Guerrero R, Toro M (2013) Interactions between a root-knot nematode (*Meloidogyne exigua*) and arbuscular mycorrhizae in coffee plant development (*Coffea arabica*). Am J Plant Sci 4:19–23

Allen MF (2011) Linking water and nutrients through the vadose zone: a fungal interface between the soil and plant systems: linking water and nutrients through the vadose zone: a fungal interface between the soil and plant systems. J Arid Land 3:155–163

Anandaraj M, Ramana KV, Sharma, YR (1990) Interaction between vesicular arbuscular mycorrhizal fungi and *Meloidogyne incognita* in black pepper. In: Bagyaraj DJ, Manjunath A (eds) Mycorrhizal symbiosis and plant growth, Proc Sec Nat Conf on Mycorrhiza, 21–23 November, Banglore, India, pp 110–112

Atilano RA, Menge JA, Vangundy SD (1981) Interaction between *Meloidogyne arenaria* and *Glomus fasciculatus* in grape. J Nematol 13:52–57

Atkinson D, Berta G, Hooker JE (1994) Impact of mycorrhizal colonisation on root architecture, root longevity and the formation of growth regulators. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel, pp 89–99

Auge RM, Toler HD, Saxton AM (2014) Arbuscular mycorrhizal symbiosis and osmotic adjustment in response to NaCl stress: a meta-analysis. Front Plant Sci 5:562–574

Azcon-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological controlof soil-borne plant pathogens – An overview of the mechanisms involved. Mycorrhiza 6:457–464

Azcon-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. Birkhäuser, Basel, pp 187–197

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Bagyaraj DJ (2014) Mycorrhizal fungi. Proc Ind Nat Sci Acad 80:415–428

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

Balestrini R, Lumini E, Borriello R, Bianciotto V (2015) Plant-soil biota interactions. In: Paul EA (ed) Soil microbiology, ecology and biochemistry. Academic Press/Elsevier, Burligton, MA/Oxford, pp 311–338

Bansal M, Mukerji KG (1994) Positive correlation between VAM induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5:39–44

Bartlem DG, Jones MGK, Hammes UZ (2014) Vascularization and nutrient delivery at root-knot nematode feeding sites in host roots. J Exp Bot 65:1789–1798. https://doi.org/10.1093/jxb/ert415

Benhamou N, Fortin JA, Hamel C, St-Arnaud M, Shatilla A (1994) Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp *chrysanthemi*. Phytopathol 84:958–968

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016) Arbuscular mycorrhizal fungi as natural biofertilizers: Let's benefit from past successes, review. Front Microbiol 6:1559. https://doi.org/10.3389/fmicb.2015.01559

Berta G, Trotta A, Fusconi A, Hooker M, Munro D, Atkinson M, Govionetti S, Morini P, Fortuna B, Tisserant V, Gianinazzi-Pearson V, Gianinazzi S (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*. Tree Physiol 15:281–293

Berta G, Sampo S, Gamalero E, Massa N, Lemanceau P (2005) Suppression of *Rhizoctonia* root-rot of tomato by *Glomus mossae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. Euro J Plant Pathol 111:279–288

Bharadwaj DP, Sadhna Alström S, Lundquist P (2012) Interactions among *Glomus irregulare*, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions. Mycorrhiza 22:437–447

Bodker L, Kjoller R, Rosendahl S (1998) Effect of phosphate and arbuscular mycorrhizal fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. Mycorrhiza 8:169–174

Borah A, Phukan PN (2003) Effect of interaction of *Glomus fasciculatum* and *Meloidogyne incognita* on growth of brinjal. Ann Plant Prot Sci 11:352–354

Borowicz VA (2001) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068

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

Carling DE, Roncadori RW, Hussey RS (1996) Interactions of arbuscular mycorrhizae, *Meloidogyne arenaria*, and phosphorus fertilization on peanut. Mycorrhiza 6:9–13

Caron M, Fortin JA, Richard C (1986) Effect of inoculation sequence on the interaction between *Glomus intraradices* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes. Can J Plant Pathol 8:12–16

Castellanos-Morales V, Keiser C, Cardenas-Navarro R, Grausgruber H, Glauninger J, Garcia-Garrido JM, Steinkellner S, Sampedro I, Hage-Ahmed K, Illana A, Ocampo JA, Vierheilig H (2011) The bioprotective effect of AM root colonization against the soil-borne fungal pathogen *Gaeumannomyces graminis* var. *tritici* in barley depends on the barley variety. Soil Biol Biochem 43:831–834

Citernesi AS, Fortuna P, Filippi C, Bagnoli G, Giovannetti M (1996) The occurrence of antagonistic bacteria in *Glomus mosseae*pot cultures. Agronomie 16:671–677

Cordier C, Gianinazzi S, Gianinazzi-Pearson V (1996) Colonisation patterns of root tissues by fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11:1017–1028

Curtis R, Robinson A, Perry R (2009) Hatch and host location. In: Perry RN, Moens M, Starr JL (eds) Root-knot Nematodes. CAB International, Wallingford, pp 139–162

Davis RM (1980) Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* root rot of citrus. Plant Dis 64:839–840

Davis RM, Menge JA (1980) Influence of *Glomus fasciculatus* and soil phosphorus on *Phytophthora* root rot of citrus. Phytopathol 70:447–452

Declerck S, Risede JM, Rufyikiri G, Delvaux B (2002) Effects of arbuscular mycorrhizal fungi on the severity of root rot of bananas caused by *Cylindrocladium spathiphylli*. Plant Pathol 51:109–115

Dehne HW (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathol 72:1115–1119

Diederichs C (1987) Interaction between five endomycorrhizal fungi and root-knot nematode and *Meloidogyne hapla* on *Allium cepa* in organic soils. J Nematol 17:55–60

Diedhiou PM, Hallmann J, Oerke EC, Dehne HW (2003) Effects of arbuscular mycorrhizal fungi and a non-pathogenic *Fusarium oxysporum* on *Meloidogyne incognita* infestation of tomato. Mycorrhiza 13:199–204

Doley K, Jite PK (2013) Management of stem-rot of groundnut (*Arachis hypogaea* L.) cultivar in field. Not Sci Biol 5:316–324

Dos Anjos ÉCT, Cavalcante UMT, Gonçalves DMC, Pedrosa EMR, dos Santos VF, Maia LC (2010) Interactions between an arbuscular mycorrhizal fungus (*Scutellospora heterogama*) and the root-knot nematode (*Meloidogyne incognita*) on sweet passion fruit (*Passiflora alata*). Braz Arch Biol Technol 53:801–809

Dugassa GD, von Alten H, Schonbeck F (1996) Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L infected by fungal pathogens. Plant Soil 185:173–182

Dumas-Gaudot E, Gollotte A, Cordier C, Gianinazzi S, Gianinazzi-Pearson V (2000) Modulation of host defense systems. In: Kapulnik Y, Douds DD Jr (eds) Arbuscular mycorrhizas: physiology and function. Kluwer, Dordrecht, pp 173–200

Elsen A, Declerck S, De Wasele D (2002) Effects of three arbuscular mycorrhizal fungi on root knot nematode (*Meloidogyne* spp.) infection of *Musa*. Infomusa 11:21–23

Elsen A, Baimey H, Swennen R, De Waele D (2003a) Relative mycorrhizal dependency and mycorrhiza-nematode interaction in banana cultivars (*Musa* spp.) differing in nematode susceptibility. Plant Soil 256:303–313

Elsen A, Beeterens R, Swennen R, De Waele D (2003b) 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, Declerck S, De Waele D (2003c) Use of root organ cultures to investigate the interaction between *Glomus intraradices* and *Pratylenchus coffeae*. Appl Environ Microbiol 69:4308–4311

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

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Fiorilli V, Catoni M, Francia D, Cardinale F, Lanfranco L (2011) The arbuscular mycorrhizal symbiosis reduces disease severity in tomato plants infected by *Botrytis cinerea*. J Plant Pathol 93:237–242

Fritz M, Jakobsen I, Lyngkjaer MF, Thordal-Christensen H, Pons-Kühnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16:413–419

Fusconi A (2014) Regulation of root morphogenesis in arbuscular mycorrhizae: what role do fungal exudates, phosphate, sugars and hormones play in lateral root formation? Ann Bot 13:19–33

Fusconi A, Gnavi E, Trotta A, Berta G (1999) Apical meristems of tomato roots and their modifications induced by arbuscular mycorrhizal and soilborne pathogenic fungi. New Phytol 142:505–516

Gamalero E, Pivato B, Bona E, Copetta A, Avidano L, Lingua 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 Int J Deal Asp Plant Biol 144:582–591

García-Chapa M, Batlle A, Laviña A, Camprubí A, Estaún V, Calvet C (2004) Tolerance increase to pear decline phytoplasma in mycorrhizal OHF-333 pear rootstock. Acta Hortic (ISHS) 657:437–441

García-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386

Gernns H, Von Alten H, Poehling HM (2001) Arbuscular mycorrhiza increased the activity of a biotrophic leaf pathogen-is a compensation possible? Mycorrhiza 11:237–243

Gheysen G, Mitchum MG (2011) How nematodes manipulate plant development pathways for infection. Curr Opin Plant Biol 14:415–421. https://doi.org/10.1016/j.pbi.2011.03.012

Gianinazzi-Pearson V, Gollotte A, Dumas-Gaudot E, Franken P, Gianinazzi S (1994) Gene expression and molecular modifications associated with plant responses to infection by arbuscular mycorrhizal fungi. In: Daniels M, Downic JA, Osbourn AE (eds) Advances in molecular genetics of plant-microbe interactions. Kluwer, Dordrecht, pp 179–186

Giri B, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmuller R, Varma A (2005) Mycorrhizosphere: strategies and functions. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Heidelberg, pp 213–252

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

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, Heidelberg, pp 3–28

Graham JH (2001) What do root pathogens see in mycorrhizas? New Phytol 149:357–359

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

Hao Z, Fayolle L, van Tuinen D, Chatagnier O, Xiaolin L, Gianinazzi S, Gianinazzi-Pearson V (2012) Local and systemic mycorrhiza-induced protection against the ectoparasitic nematode

Xiphinema index involves priming of defence gene responses in grapevine. J Exp Bot 63:3657–3672
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
Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42
Hause B, Schaarschmidt S (2009) The role of jasmonates in mutualistic symbioses between plants and soil-borne microorganisms. Phytochem 70:1589–1599
Hause B, Mrosk C, Isayenkov S, Strack C (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochem 68:101–110
Hiltner L (1904) Uber neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderden berucksichtigung und Brache. Arb Dtsch Landwirtsch Gesellschaft 98:59–78
Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil 321:153–187
Hol WHG, Cook R (2005) An overview of arbuscular mycorrhizal fungi-nematode interactions. Basic Appl Ecol 6:489–503
Hooker JE, Jaizme-Vega M, Atkinson D (1994) Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel, pp 191–200
Hussey R, Roncadori R (1978) Interaction of *Pratylenchus brachyurus* and *Gigaspora margarita* on cotton. J Nematol 10:16–20
Ijdo M, Cranenbrouck S, Declerck S (2011) Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21:1–16
Jabaji-Hare SH, Stobbs LW (1984) Electron microscopic examination of tomato roots coinfected with *Glomus* sp. and tobacco mosaic virus. Phytopathol 74:277–279
Jain RK, Sethi CL (1988) Influence of endomycorrhizal fungi *Glomus fasciculatum* and *G. epigaeus* on penetration and development of *Heterodera cajani* on cowpea. Ind J Nematol 18:89–93
Jaiti F, Kassami M, Meddich A, El Hadrami I (2008) Effect of arbuscular mycorrhization on the accumulation of hydroxycinnamic acid derivatives in date palm seedlings challenged with *Fusarium oxysporum* f. sp. *albedinis*. J Phytopathol 156:641–646
Jayaram J, Kumar D (1995) Influence of mungbean yellow mosaic virus on mycorrhizal fungi associated with *Vigna radiata* var. PS 16. Ind Phytopathol 48:108–110
Jones DL, Hodge A, Kuzyakov Y (2004) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480
Jones JT, Haegeman A, Danchin EGJ, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-López R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961
Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ (2012) Mycorrhiza-induced resistance and priming ofplant defenses. J Chem Ecol 38:651–664
Kamińska M, Klamkowski K, Berniak H, Sowik I (2010) Response of mycorrhizal periwinkle plants to aster yellows phytoplasma infection. Mycorrhiza 20:161–166
Kantharaju V, Krishnappa K, Ravichardra NG, Karuna K (2005) Management of root-knot nematode, *Meloidogyne incognita* on tomato by using indigenous isolates of AM fungus, *Glomus fasciculatum*. Ind J Nematol 35:32–36
Kareem TA, Hassan MS (2014) Evaluation of *Glomus mosseae* as biocontrol agents against *Rhizoctonia solani* on Tomato. J Biol Agric Healthcare 4:15–19
Khaosaad T, García-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734
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

Koffi MC, Vos C, Draye X, Declerck S (2013) Effects of *Rhizophagus irregularis* MUCL 41833 on the reproduction of *Radopholus similis* in banana plantlets grown under in vitro culture conditions. Mycorrhiza 23:279–288. https://doi.org/10.1007/s00572-012-0467-6

Koide RT, Schreiner RP (1992) Regulation of the vesicular arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 43:557–581

Kotcon JB, Bird GW, Rose LM, Dimoff K (1985) Influence of *Glomus fasciculatum* and *Meloidogyne hapla* on *Allium cepa* in organic soils. J Nematol 17:55–60

Labeena P, Sreenivasa MN, Lingaraju S (2002) Interaction effects between arbuscular mycorrhizal fungi and root-knot nematode *Meloidogyne incognita* on tomato. Ind J Nematol 32:118–120

Larsen J, Ravnskov S, Jakobsen I (2003) Combined effect of an arbuscularmycorrhizal fungus and a biocontrol bacterium against *Pythium ultimum* in soil. Folia Geobotanica 38:145–154

Lee CS, Lee YJ, Jeun YC (2005) Observations of infection structures on the leaves of cucumber plants pre-treated with arbuscular mycorrhiza *Glomus intraradices* after challenge inoculation with *Colletotrichum orbiculare*. Plant Pathol J 21:237–243

Li HY, Yang GD, Shu HR, Yang YT, Ye BX, Nishida I, Zheng CC (2006) Colonization by the arbuscular mycorrhizal fungus *Glomus versiforme*induces a defense response against the root-knot nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr), which includes transcriptional activation of the class III chitinase gene VCH3. Plant Cell Physiol 47:154–163

Li HY, Yanagi A, Miyawaki Y, Okada T, Matsubara Y (2010) Disease tolerance and changes in antioxidative abilities in mycorrhizal strawberry plants. J Jpn Soc Hortic Sci 79:174–178

Linderman RG (1988) Mycorrhizal Interactions with the rhizosphere microflora–the mycorrhizosphere effect. Phytopathol 78:366–371

Linderman RG (1994) Role of VAM fungi in biocontrol. In: Pfleger FL, Linderman RG (eds) Mycorrhizae and plant health. APS, St Paul, pp 1–26

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

Lioussanne L (2010) The role of the arbuscular mycorrhiza-associated rhizobacteria in the biocontrol of soilborne phytopathogens. Span J Agric Res 8(S1):S51–S61

Lioussanne L, Jolicoeur 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

Liu RJ (1995) Effect of vesicular-arbuscular mycorrhizal fungi on Verticillium wilt of cotton. Mycorrhiza 5:293–297

Liu RJ, Li HF, Shen CY, Chiu WF (1995) Detection of pathogenesis-related proteins in cotton plants. Physiol Mol Plant Pathol 47:357–363

Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529–544

Lopez-Raez JA, Verhage A, Fernandez I, Gracia JM, Azcon-Aguilar C, Flors V (2010) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 61:2589–2601

MacGuidwin A, Bird G, Safir G (1985) Influence of *Glomus fasciculatum* on *Meloidogyne hapla* infecting *Allium cepa*. J Nematol 17:389–395

Maffei G, Miozzi L, Fiorilli V, Novero M, Lanfranco L, Accotto GP (2014) The arbuscular mycorrhizal symbiosis attenuates symptom severity and reduces virus concentration in tomato infected by Tomato yellow leaf curl Sardinia virus (TYLCSV). Mycorrhiza 24:179–186

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748

Malik RJ, Dixon MH, Bever JD (2016) Mycorrhizal composition can predict foliar pathogen colonization in soybean. Biol Control 103:46–53

Marro N, Lax P, Cabello M, Doucet ME, Becerra AG (2014) Use of the arbuscular mycorrhizal fungus *Glomus intraradices* as biological control agent of the nematode *Nacobbus aberrans* parasitizing tomato. Braz Arch Biol Technol 57:668–675

Masadeh B, von Alten H, Grunewaldt-Stoecker G, Sikora RA (2004) Biocontrol of root knot nematodes using the arbuscular mycorrhizal fungus *Glomus intraradices* and the antagonistic *Trichoderma viridae* in two tomato cultivars differing in their suitability as hosts for the nematodes. J Plant Dis Prot 111:322–333

McArthur DA, Knowles NR (1992) Resistance responses of potato to vesicular-arbuscular mycorrhizal fungi under varying abiotic phosphorus levels. Plant Physiol 100:341–351

Miller RM, Reinhardt DR, Jastrow JD (1995) External hyphalproduction of vesicular-arbuscular mycorrhizal fungi in pastureand tallgrass prairie communities. Oecologia 103:17–23

Miozzi L, Catoni M, Fiorilli V, Philip MM, Accotto GP, Lanfranco L (2011) Arbuscular mycorrhizal symbiosis limits foliar transcriptional responses to viral infection and favors long-term virus accumulation. Mol Plant Microbe Interact 24:1562–1572

Miransari M, Smith DL (2014) Plant hormones and seed germination. Environ Exp Bot 99:110–121

Mukherjee KG, Ane JM (2011) Germinating spore exudates from arbuscular mycorrhizal fungi: molecular and developmental responses in plants and their regulation by ethylene. Mol Plant Microbe Interact 24:260–270

Naglaa ASM, Essa TA, Manal AHE, Kamel SM (2016) Efficacy of free and formulated arbuscular mycorrhiza, *Trichoderma viride* and *Pseudomonas fluorescens* on controlling tomato root rot diseases. Egypt J Biol Pest Control 26:477–486

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. https://doi.org/10.3389/fpls.2016.01574

Nehra S (2004) VAMF and organic amendments in the management of *Meloidogyne incognita* infected ginger. J Ind Bot Soc 83:90–97

Nemec S, Myhre D (1984) Virus-*Glomus etunicatum* interactions in Citrus rootstocks. Plant Dis 68:311–314

Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, Tahna Maafi Z (2011) Current nematode threats to world agriculture. In: Jones J, Gheysen G, Fenoll C (eds) Genomics and molecular genetics of plant-nematode interactions. Springer, Heidelberg, pp 21–44

Norman JR, Hooker JE (2000) Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. Mycol Res 104:1069–1073

Norman J, Atkinson D, Hooker J (1996) Arbuscular mycorrhizal fungal-induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. Plant Soil 185:191–198

O'Bannon J, Nemec S (1979) The response of Citrus limon seedlings to a symbiont, *Glomus etunicatus*, and a pathogen, *Radopholus similis*. J Nematol 11:270–275

O'Bannon J, Inserra R, Nemec S, Vovlas N (1979) The influence of *Glomus mosseae* on *Tylenchulus semipenetrans*-infected and uninfected *Citrus limon* seedlings. J Nematol 11:247–250

Olah B, Briere C, Becard G, Denarie J, Gough C (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J 44:195–207

Ozgonen H, Erkilic A (2007) Growth enhancement and Phytophthora blight (*Phytophthora capsici* L.) control by arbuscular mycorrhizal fungal inoculation in pepper. Crop Prot 26:1682–1688

Pandey R (2005) Field application of bio-organics in the management of *Meloidogyn incognita* in *Mentha arvensis*. Nematol Mediterr 33:51–54

Paszkowski U, Boller T (2002) The growth defect of *lrt1*, a maize mutant lacking lateral roots, can be complemented by symbiotic fungi or high phosphate nutrition. Planta 214:584–590

Pereira JAP, Vieira IJC, Freitas MSM, Prins CL, Martins MA, Rodrigues R (2016) Effects of arbuscular mycorrhizal fungi on *Capsicum* spp. J Agric Sci 154:828–849

Perry RN, Moens M (2011) Introduction to plant-parasitic nematodes; modes of parasitism. In: Jones JT, Gheysen L, Fenoll C (eds) Genomics and molecular genetics of plant–nematode interactions. Springer, Heidelberg, pp 3–20

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

Pineda A, Zheng SJ, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci 15:507–514

Pinochet J, Calvet C, Camprubí A, Fernández C (1996) Interactions between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in perennial crops: a review. Plant Soil 185:183–190

Pinochet J, Fernandez C, de Jaimez M, Tenoury P (1997) Micropropagated banana infected with *Meloidogyne javanica* responds to *Glomus intraradices* and phosphorus. Hortic Sci 32:35–49

Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Pozo MJ, Azcón-Aguilar C, Dumas-Gaudot E, Barea JM (1999) ß-1,3-glucanase activities in tomato roots inoculated witharbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. Plant Sci 141:149–157

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C (2002) Localized verses systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot 53:525–534

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. Springer, Dordrecht, pp 193–207

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, Dordrecht, pp 1–7

Rao MS, Kerry BR, Gowen SR, Bourne JM, Reddy PP (1997) Management of *Meloidogyne incognita* in tomato nurseries by integration of *Glomus deserticola* with *Verticillium chlamydosporium*. J Plant Dis Prot 104:419–422

Rao MS, Reddy PP, Mohandas MS (1998) Bio-intensive management of *Meloidogyne incognita* on eggplant by integrating *Paecilomyces lilacinus* and *Glomus mosseae*. Nematol Mediterr 26:213–216

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred million year old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci USA 91:11841–11843

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

Ronsheim ML (2016) Plant genotype influences mycorrhiza benefits and susceptibility to a soil pathogen. Am Midl Nat 175(1):103–112

Rosendahl CN, Rosendahl S (1990) The role of vesicular arbuscular mycorrhizal fungi in controlling damping-off and growth reduction in cucumber caused by *Pythium ultimum*. Symbiosis 9:363–366

Sankaranarayanan C, Sundarababu R (1997) Effect of oil cakes and nematicides on the growth of blackgram (*Vigna mungo*) inoculate with VAM fungus (*Glomus fasciculatum*) and root-knot nematode (*Meloidogyne incognita*). Ind J Nematol 27:128–130

Sankaranarayanan C, Sundarababu R (2010) Influence of application methods of arbuscular mycorrhiza *Glomus mosseae* in the bio-management of root knot nematode, *Meloidogyne incognita* on black gram (*Vigna mungo* L.) Hepper. J Biol Control 24:51–57

Scannerini S, Fusconi A, Mucciarelli M, Seckback J (2001) The effect of endophytic fungi on host plant morphogenesis. In: Seckback J (ed) Symbiosis: organisms and model systems. Kluwer, Dordrecht, pp 427–447

Scheffknecht S, Mammerler R, Steinkellner S, Vierheilig H (2006) Root exudates of mycorrhizal tomato plants exhibit a different effect on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants. Mycorrhiza 16:365–370

Scheffknecht S, St-Arnaud M, Khaosaad T, Steinkellner S, Vierheilig H (2007) An altered root exudation pattern through mycorrhization affecting microconidia germination of the highly specialized tomato pathogen *Fusarium oxysporum* f.sp *lycopersici* (Fol) is not tomato specific but also occurs in *Fol* nonhost plants. Can J Bot 85:347–352

Schellenbaum L, Berta G, Raviolanirina F, Tisserant B, Gianinazzi S, Fitter AH (1991) Influence of endomycorrhizal infection on root morphology in a micropropagated woody plant species (*Vitis vinifera* L.) Ann Bot 68:135–141

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

Schüßler AH, Gehrig H, Schwarzott D, Walker C (2001) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. Mycol Res 105:5–15

Shakoor S, Inam-ul-Haq M, Bibi S, Ahmed R (2015) Influence of root inoculations with vasicular arbuscular mycorrhizae and rhizomyx for the management of root rot of chickpea. Pak J Phytopathol 27:153–158

Sharma HKP, Mishra SD (2003) Effect of plant growth promoter microbes on root knot nematode *Meloidogyne incognita* on okra. Curr Nematol 14:57–60

Sharma IP, Sharma AK (2016) Physiological and biochemical changes in tomato cultivar PT-3 with dual inoculation of mycorrhiza and PGPR against root-knot nematode. Symbiosis 69. https://doi.org/10.1007/s13199-016-0423-x

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y (1999) Mycorrhiza-induced changes in disease severity and PR protein expression in tobacco leaves. Mol Plant Microbe Interact 12:1000–1007

Siasou E, Standing D, Killham K, Johnson D (2009) Mycorrhizal fungi increase biocontrol potential of *Pseudomonas fluorescens*. Soil Biol Biochem 41:1341–1343

Siddiqui ZA, Akhtar MS (2006) Biological control of root-rot disease complex of chickpea by AM fungi. Archiv Phytopathol Plant Prot 39:389–395

Siddiqui ZA, Mahmood I (1996) Biological control of *Heterodera cajani* and *Fusarium udum* on pigeonpea by *Glomus mosseae*, *Trichoderma harzianum* and *Verticillium chlamydosporium*. Isr J Plant Sci 44:49–56

Siddiqui ZA, Singh LP (2005) Effects of fly ash and soil micro-organisms on plant growth, photosynthetic pigments and leaf blight of wheat. J Plant Dis Protect 112:146–155

Sikes BA (2010) When do arbuscular mycorrhizal fungi protect plant roots from pathogens? Plant Signal Behav 5:763–765

Singh M (2015) Interactions among arbuscular mycorrhizal fungi, *Trichoderma harzianum*, *Aspergillus niger* and biocontrol of wilt of tomato. Arch Phytopathol Plant Protect 48:205–211

Singh I (2017) Antimicrobials in higher plants: classification, mode of action and bioactivities. Chem Biol Lett 4:48–62

Singh R, Adholeya A, Mukerji KG (2000) Mycorrhiza incontrol of soil borne pathogens. In: Mukerji KG, Chamola BP, Singh J (eds) Mycorrhizal biology. Kluwer Academic/Plenum Publishers, New York, pp 173–196

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

Sipahioglu MH, Demir S, Usta M, Akkopru A (2009) Biological relationship of Potato virus Y and arbuscular mycorrhizal fungus *Glomus intraradices* in potato. Pest Tech 3:63–66

Sitaramaiah K, Sikora RA (1982) Effect of mycorrhizal fungus *Glomus fasciculatum* on the host parasite relationship of *Rotylenchulus reniformis* in tomato. Nematologica 28:412–419

Smith GS, Kaplan DT (1988) Influence of mycorrhizal fungus, phosphorus and burrowing nematode interactions on growth of rough lemon citrus seedlings. J Nematol 20:539–544
Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London
Smith SE, Read DJ (2008) Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis. Academic Press, London, pp 145–148
Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystems scales. Annu Rev Plant Biol 63:227–250
Sood GS (2003) Chemotactic response of plant-growth-promoting bacteria towards roots of vesicular-arbuscular mycorrhizal tomato plants. FEMS Microbiol Ecol 45:219–227
St-Arnaud M, Vujanovic V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases andpests. In: Hamel C, Plenchette C (eds) Mycorrhizaein crop production. Haworth Press, Binghamton, pp 67–122
Steinkellner S, Lendzemo 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
Stoffelen R, Verlinden R, Xuyen NT, De Waele D, Swennen R (2000) Host plant response of Eumusa and *Australimusa bananas* (*Musa* spp.) to migratory endoparasitic and root-knot nematodes. Nematol 2:907–916
Strobel N, Hussey R, Roncadori R (1982) Interactions of vesicular-arbuscular mycorrhizal fungi, *Meloidogyne incognita*, and soil fertility on peach. Phytopathol 72:690–694
Tahat MM, Kamaruzaman Sijam K, Othman R (2012) The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralstonia solanacearum* under glasshouse conditions. Afr J Biotechnol 11:13085–13094
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
Trotta A, Varese GC, Gnavi E, Fusconi A, Sampo S, Berta G (1996) Interactions between the soil borne pathogen *Phytophthoranicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209
Tylka GL, Hussey RS, Roncadori RW (1991) Interactions of vesicular-arbuscular mycorrhizal fungi, phosphorus and *Heterodera glyciens* on soybean. J Nematol 23:122–123
Van Wees SC, Van der Ent S, Pieterse CM (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448
Veresoglou SD, Rillig MC (2012) Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. Biol Lett 8:214–217
Vierheilig H, Steinkellner S, Khaosaad T (2008) The biocontrol effect of mycorrhization on soilborne fungal pathogens and the autoregulation of the AM symbiosis: one mechanism, two effects? In: Varma A (ed) Mycorrhiza. Springer, Berlin, pp 307–320
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 Physiol 49:509–514
Vos C (2012) Arbusculaire Mycorrhizenschimmels in De Biocontrole Van Plantenparasitaire Nematoden. University of Leuven (KU Leuven), Leuven
Vos C, Schouteden N, Tuinen D, Chatagnier O, Elsen A, De Waele D, Panis B, Gianinazzi-Pearson V (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
Vos CM, Yang Y, De Coninck B, Cammue BPA (2014) Fungal (-like) biocontrol organisms in tomato disease control. Biol Control 74:65–81
Waceke JW, Waudo SW, Sikora R (2001) Suppression of *Meloidogyne hapla* by arbuscular mycorrhiza fungi (AMF) on pyrethrum in Kenya. Int J Pest Manag 47:135–140
Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53:197–201

Wesemael W, Viaene N, Moens M (2011) Root-knot nematodes (*Meloidogyne* spp.) in Europe. Nematol 13:3–16. https://doi.org/10.1163/138855410X526831

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

Wyss U (2002) Feeding behaviour of plant-parasitic nematodes. In: Lee DL (ed) Biology of nematodes. Taylor and Francis, London, pp 233–259

Xavier LJC, Boyetchko SM (2004) Arbuscular mycorrhizal fungi in plant disease control. In: Arora D, Bridge P, Bhatnagar D (eds) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, New York, pp 183–194

Yang H, Zhang Q, Dai Y, Liu Q, Tang J, Bian X (2014) Effects of arbuscular mycorrhizal fungi on plant growth depend on root system: a meta-analysis. Plant Soil 389:361–374

Yao M, Tweddell R, Désilets H (2002) Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. Mycorrhiza 12:235–242

Yuanjing L, Zhilei L, Hongyan H, Hong L, Xiancan Z, Xuhui L, Chunjie T (2013) Arbuscular mycorrhizal fungi-enhanced resistance against *Phytophthora sojae* infection on soybean leaves is mediated by a network involving hydrogen peroxide, jasmonic acid, and the metabolism of carbon and nitrogen. Acta Physiol Plant 35:3465–3475

Zhu HH, Yao Q (2004) Localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. J Phytopathol 152:537–542

Chapter 8
Mycorrhizal Fungi as Control Agents Against Plant Pathogens

Swati Tripathi, Siddhartha Kumar Mishra, and Ajit Varma

Abstract Biofertilizers comprise single or consortia of living microorganisms which are responsible for the direct or indirect benefits rendered to growth of various plants. These microbial inoculants are produced from cultures of certain soil organisms that can improve soil fertility and crop productivity. They solubilise phosphorous, fix atmospheric nitrogen, oxidize sulfur, decompose organic material and alter the dynamics and properties of soil resulting in various benefits to plant growth and crop production. Biofertilizers help to increase access to nutrients thus providing growth-promoting factors for plants. This increased availability and efficient absorption of nutrients stimulates plant growth by hormone action and improves crop yield. One of the most abundant fungi in agricultural soil, the arbuscular mycorrhizal (AM) fungi, play a very important role as biofertilizers. They form mutualistic relationships with roots of 90% of plants, promote absorption of nutrients and water, control plant diseases, and improve soil structure. Plants colonized by mycorrhizae grow better than those without them and are beneficial in natural and agricultural systems. The use of AM fungi as biofertilizers is not new; they have been produced for use in agriculture, horticulture, landscape restoration, and soil remediation for almost two decades.

8.1 Introduction

Mycorrhiza refers to associations or symbioses between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth. The term mycorrhiza (modern Latin of Greek words *mykes* + fungi, *rhiza* + root) which literally means 'fungus-root' was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank (Trappe 2005). Generally, these symbioses are often characterized by bi-directional exchange of

S. Tripathi (✉) • A. Varma
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3, Fourth Floor, Noida 201313, India
e-mail: stripathi2@amity.edu

S.K. Mishra
School of Biological Sciences, Dr. Harisingh Gour Central University, Sagar 470003, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_8

plant-produced carbon to the fungus and fungal-acquired nutrients to the plant thereby providing a critical linkage between the plant root and soil. All mycorrhizal associations are symbiotic, but some are not mutualistic (Brundrett 2004). A vast majority of terrestrial as well as aquatic plants have been reported to form symbiotic associations with fungi (Christie et al. 2004; Willis et al. 2013).

The formation of symbiotic association with mycorrhizae significantly changes the physiology and/or morphology of roots and plants in general (Linderman 1988), leading to altered root exudation (Bansal and Mukerji 1994). The changes in root exudates affect the microbial communities around the roots, leading to the formation of 'mycorrhizosphere' which is the zone of soil where the physical, chemical and microbiological processes are influenced by exudates of plant roots and their associated mycorrhizal fungi (Ames 1987; Varma and Hock 1998; Giri et al. 2005). Mycorrhizal plants are often more competitive and exhibit enhanced tolerance against biotic and abiotic stresses compared to non-mycorrhizal plants (Marler et al. 1999; Mukerji et al. 2000; Peterson and Massicotte 2004; Zhang et al. 2011). The benefits afforded to the plants from mycorrhizal symbioses can be characterized agronomically by increased growth, yield and ecologically by improved fitness (i.e., reproductive ability). These benefit range from absorption of toxic elements from the soil such as heavy metals to soil restoration, establishment of green cover, disease resistance, drought tolerance etc. (Evelin et al. 2009; Miransari 2010). These play a significant role in sustaining the ecosystem by enriching the soil and providing nutrition to the plant for better growth, by sequestering the nutrients from the soil, translocating it to the plant and in turn achieving carbon source from the plant (Rooney et al. 2011; Shi et al. 2013). This in turn reduces the dependence if not eliminate external chemical inputs and makes the utilization of the soil nutrient highly efficient and makes them extremely important for the regions where large chunks of land is degraded and rendered unfertile for cultivation. Given that the majority of cultivars used for human and animal food purposes are colonized by mycorrhizae, we can consider utilizing this symbiosis for the benefit of agriculture, by selecting the best plant-fungus combinations. It is then possible to promote sustainable agriculture through healthier cropping systems and reduction of use of chemical inputs (pesticides, fertilizers), while ensuring crop profitability and environmental quality. Use of AM fungi in the long term would thus favor an agricultural system that is both production and protection oriented thus enhancing stabilizing of agro-ecosystems.

8.2 Diversity of Mycorrhizal Fungi

Although mycorrhizae were discovered 200 years ago, they were not seriously included in the multidisciplinary science of biology until the 1980s. Mycorrhizae form mutualistic symbiotic relationships with plant roots of more than 80% of land plants including many important crops and forest tree species and 92% land plant families (Smith and Zhu 2001; Gentili and Jumpponen 2006; Wang and Qiu 2006;

Rinaldi et al. 2008; Prasad et al. 2017). More than 6000 fungal species are capable of establishing mycorrhizae approximately with 2,40,000 plant species (Sharma 2001).

Early morphological classifications separated mycorrhizae into endomycorrhizal, ectomycorrhizal and ect-endomycorrhizal associations based on the relative location of fungi in and on roots (Peyronel et al. 1969). These three types were not enough to describe the diversity of mycorrhizal associations. Brundrett (2004) recommended that mycorrhizal associations were defined and classified primarily by anatomical criteria regulated by the host plant. A revised classification scheme for types and categories of mycorrhizal associations defined main categories of arbuscular mycorrhizal associations (AM) as 'linear' or 'coiling', and of ectomycorrhizal associations (ECM) as 'epidermal' or 'cortical'. Subcategories of coiling AM and epidermal ECM occur in certain host plants. Fungus-controlled features resulted in 'morphotypes' within categories of AM and ECM. Following this classification, arbutoid and monotropoid associations had been considered subcategories of epidermal ECM and ectendomycorrhizas were relegated to an ECM morphotype. Harley and Smith (1983) and Friberg (2001) recognized seven types that, for the most part, still comprise the generally accepted classification. These include Ectomycorrhizae, Endomycorrhizae, Ect-endomycorrhizae, Arbutoid mycorrhizae, Monotropoid mycorrhizae and Orchid mycorrhizae (Raina et al. 2000; Gentili and Jumpponen 2006; Das et al. 2007; Tao et al. 2008; Zhu et al. 2008) (Fig. 8.1; c.f. Das et al. 2007). However, different people use different criteria and hence describe different types and categories of mycorrhizal associations. The following terms are most commonly used in the mycorrhizal studies.

8.2.1 Ectomycorrhizae (ECM)

The characteristic feature of ectomycorrhizae ("outside" mycorrhizae) is the presence of hyphae between root cortical cells producing a netlike structure called the 'Hartig net' (Scheidegger and Brunner 1993). Hyphae of the Hartig's net completely envelope the host cells to provide maximum contact between host and fungus. The Hartig's net exhibits a complex labyrinthine growth mode with finger-like structures termed palmettes and with rare hyphal septations (Blasius et al. 1986).

8.2.2 Endomycorrhizae

Endomycorrhizae ("inside" mycorrhizae) grow within cortical cells and do not form a mantle around the root, but instead the fungal hyphae establish between the cortex cells, and often enter them. Mycelia do not enter the vascular system.

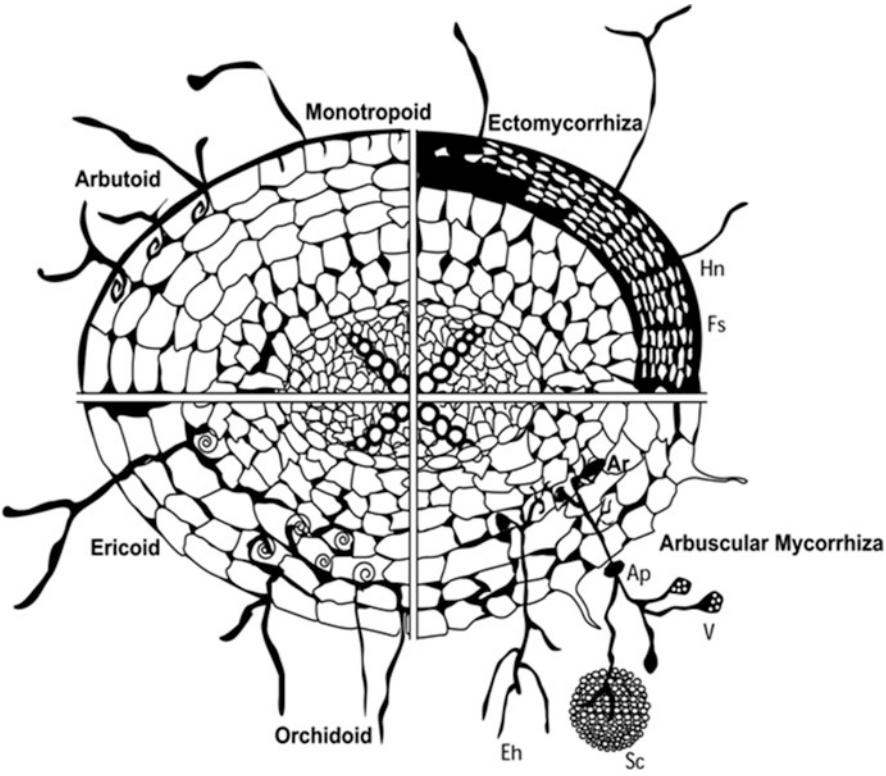


Fig. 8.1 Types of mycorrhizal fungi, *Fs* fungal sheath; *Eh* extramatrical hyphae, *Eh* extramatrical hyphae, *Hn* hartig's, *V* vesicle, *Sc* sporocarp, *Ap* appresorium, *Ar* arbuscule, *Sc* sporocarp, *Ap* appresorium (c.f. Das et al. 2007)

8.2.3 Arbuscular Mycorrhizae (AM)

It is a member of endomycorrhizae. The diagnostic feature of arbuscular mycorrhizae (AM) is the development of a highly branched arbuscule within root cortical cells. The fungus initially grows between cortical cells, but soon penetrates the host cell wall and grows within the cell. As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment where material of high molecular complexity is deposited. This apoplastic space/compartment prevents direct contact between the plant and fungus cytoplasm and allows for efficient transfer of nutrients between the symbionts. The arbuscules are relatively short lived, less than 15 days. The fungi that form AM were all classified as members of the order *Glomales*, which was further subdivided into suborders based on the presence or absence of vesicles. Schüßler et al. (2001) described a new phylum *Glomeromycota* which includes AMF. AM can be divided into two main types, the *Arum*-type and the *Paris*-type (Smith and Smith 1997). In the *Arum*-type, usually

one arbuscule develops through repeated branching of a hypha that penetrates through the cortical cell wall (Bonfante and Perotto 1995) whereas in *Paris*-type, penetration of the cortical cell wall by a single hypha is followed by extensive coiling of this hypha from which lateral branches are initiated to form arbusculate coils (Cavagnaro et al. 2001). Originally, the term 'vesicular-arbuscular mycorrhiza' (VAM) was applied to symbiotic associations formed by all Glomeromycota mycorrhizal fungi. However, since a major proportion of fungi lacks the ability to form the vesicles in roots, AM is now the preferred acronym (Varma and Hock 1998).

8.2.4 Ect-endomycorrhizae

The ect-endomycorrhizae form typical ECM structures, except that the mantle is thin or lacking and hyphae in the Hartig net may penetrate root cortical cells. The ect-endomycorrhizae is replaced by ECM as the seedling matures.

8.2.5 Ericaceous Mycorrhizae

The term ericaceous is applied to mycorrhizal associations found in plants of the order *Ericales*. The hyphae in the root can penetrate cortical cells (endomycorrhizal habit); however, no arbuscules are formed.

8.2.6 Orchidaceous Mycorrhizae

The association between orchids and mycorrhizal fungi is included in this category. These fungi enter plant cells by invaginating the cell membrane and forming hyphal coils within cells of the protocorm and developing root. These coils are active for only a few days, after which they loose turgor and degenerate while nutrient contents are absorbed by the developing orchid. The fungi participating in this type of symbiosis are basidiomycetes similar to those involved in decaying wood (e.g., *Coriolus* sp., *Fomes* sp. and *Marasmius* sp.) and pathogenesis (e.g., *Armillaria* sp. and *Rhizoctonia* sp.). In mature orchids, mycorrhizae also have roles in nutrient uptake and translocation. Orchid mycorrhizae support orchid development and initial root development by delivering nutrients for germination, protocorm and initial root development (Peterson and Massicotte 2004).

8.3 Mycorrhizal Functions as Biofertilizers and Biocontrol Agents Against Plant Pathogens

Mycorrhizal fungi colonize the roots and their rhizosphere and spread out over several centimeters in the form of ramified filaments. This filamentous network dispersed inside as well as outside the roots allows the plant to have access to a greater quantity of water and soil minerals required for its nutrition. In return, the plant provides the fungus with sugars, amino acids and vitamins essential to its growth (Bonfante and Genre 2010; Smith and Smith 2012). As a result of its improved nourishment, a mycorrhiza-colonized plant has better growth. The key functions of mycorrhizal symbiosis can be summarized as follows: (1) improving rooting and plant establishment (2) enhancing plant tolerance to (biotic and abiotic) stresses; (3) improving nutrient cycling; (4) enhancing plant community diversity. Besides, they are helpful in increased photosynthesis efficiency, increased water conducting capacity, enhanced nutrient uptake, enhanced plant tolerance to environmental stresses including drought, cold, salinity and pollution, providing protection from harmful soil borne pathogens, changing the supply of mineral nutrients from soil thereby modifying soil fertility, mycorrhizosphere and aggregation of soil particles and in promoting growth, fitness and conservation of endangered plants (Giri et al. 2003; Giri and Mukerji 2004; Kapoor and Bhatnagar 2007; Goltapeh et al. 2008; Kapoor et al. 2008; Bonfante and Genre 2010; Bothe et al. 2010; Yaseen et al. 2012).

8.3.1 Plant Growth Promotion

Mycorrhizal colonization highly promotes the plants growth (Cavagnaro et al. 2006; Smith and Read 2008; Nunes et al. 2010). There are several mechanisms to explain the growth promotion due to mycorrhizal association. These include production of metabolites like amino acids, vitamins, phytohormones, and/or solubilization and mineralization processes (Azcon-Aguilar et al. 2002; Bharadwaj et al. 2008; Khan et al. 2009; Meddad-Hamza et al. 2010; Li et al. 2013). Besides providing nutritional and structural benefits, mycorrhizae also impart other benefits to plants including improved nitrogen fixation, enhanced photosynthesis rate, production/accumulation of secondary metabolites, osmotic adjustment under stress, and increased resistance against biotic and abiotic stresses (Wu and Xia 2006; Khaosaad et al. 2007; Takeda et al. 2007; Schliemann et al. 2008; Sheng et al. 2009; Ruiz-Lozano and Aroca 2010; Selvakumar and Thamizhiniyan 2011; Shinde et al. 2013). The mycorrhizal fungi symbiosis is responsible for huge fluxes of photosynthetically fixed carbon from plants to the soil. As mycorrhizae are mutualistic symbiont, they drain up to 20% of photosynthetic carbon (Jakobsen and Rosendahl 1991) and in return, provide plants with large amounts of nutrients (P, N, K, Zn etc.) and water from the soil. The carbon inflow to soil attracts soil microbes;

together producing a functionally diverse and dynamic soil biota (Schreiner et al. 1997), which is fundamental for the plant nutrition in natural systems (Friberg 2001). Mycorrhizal fungi also produce glycoprotein extracellularly on the mycelia in the bulk soil, which together with the physical network of hyphae, helps to aggregate soil (Rillig et al. 2010; Singh 2012), thus improving aeration and water percolation. This also suggests that they mediate significant inter-plant carbon transfer in nature (Bidartondo et al. 2002). They can also improve crop growth and yield by alleviating the negative influence of allelochemicals (Javaid 2009). Mycorrhizal fungi fructify abundantly and, acquire increased resistance to heavy metals, drought, and salinity, and also protect plants from pathogens (Azcon-Aguilar et al. 2002; Vivas et al. 2003; Gosling et al. 2006; Marulanda et al. 2006, 2009; Colla et al. 2008).

8.3.2 Phosphate Mobilization and Transport

Mycorrhizal plants accumulate more P than non-mycorrhizal plants (Smith et al. 2000; Burleigh et al. 2002; Chalot et al. 2002; Tibbett and Sanders 2002; Smith and Read 2008; Baslam et al. 2011). The positive role of mycorrhizal symbiosis on the use of mineral P by the host plant has been studied (Casarin et al. 2004; Smits et al. 2008 and Liu et al. 2008). Two enzymes, Acid and alkaline phosphatases (ACP and ALP) are the two forms of the phosphatases which exhibit acid and alkaline pH optima, respectively. They are ubiquitous in nature, and occur in a variety of fungi (Arnold 1981; Dhamija et al. 1987; Pedregosa et al. 1991) and plants (Ashford and Jacobson 1974; Hasegawa et al. 1976; Basha 1984). Mycorrhizal plants have been reported to show the up-regulation of the secreted acid phosphatase gene which is mainly involved in uptake of P and responsible for the improved P acquisition (Ezawa et al. 2005) while ALP has been linked with its assimilation (Plassard and Dell 2010). Mycorrhizal fungi produce extraradical hyphae that are able to explore the soil away from the root and provide a conduit to the root where exchanges between fungal and root cells occur. They considerably increase the volume of soil exploited by mycorrhizal plants thus improving the phosphate uptake by the host plant (Sharda and Koide 2010). The gradually increasing proportion of hyphae in the extra-matrical mycelium exhibits ACP activity, particularly under low P conditions, and indicates an induction of ALP activity by P limitation. These roots and the extraradical hyphae are the main production areas for ACP activities (van Aarle and Plassard 2010) and facilitate the uptake of Pi from soil solution occurs through fungal Pi transporter(s) via two types of high-affinity Pi transporters: Pi:H+ or Pi:Na+ transporters (Plassard and Dell 2010) localized at the fungal/soil interface.

8.3.3 Siderophore Production

Siderophores are metabolic product of a fungus which bind iron and facilitate its transport from the surrounding environment into the microbial cell. They are involved in the overall enhancement of Fe, Zn and Cu uptake by mycorrhizal fungi colonized plants (Clark and Zeto 1996; Caris et al. 1998; Liu et al. 2000; Lee and George 2005; Silvia et al. 2005; Schreiner 2007). Mycorrhizal fungi establish a hyphal net inside and around the roots. Both, microbes and plant roots produce siderophores and phytosiderophores respectively, which are low molecular chelators having high affinity to Fe^{3+} (Leyval and Reid 1991; Caris et al. 1998). These are secondary metabolites from inducible genes which are generally expressed by Fe^{3+} and to some extent by Zn, Cu and Mn deficiencies (Cakmak et al. 1994; Zhang et al. 1991). They readily make complexes with other metal ions such as Zn, Cu, Mn, Cd, Cr, Ni and Al (Romheld 1987; Buyer et al. 1993; Bakkaus et al. 2006).

Some fungal species are reported to produce more siderophore than bacteria (Milagres et al. 1999). Ericoid mycorrhizal fungi and ectomycorrhizal fungi, *Cenococcum geophilum* and *Hebeloma crustuliniforme* release ferricrocin or fusigen as the main siderophores. Arbuscular mycorrhizal fungi have been reported to enhance Fe-uptake rates of associated host plants which can be taken as an indication that mycorrhizal siderophores of a yet unknown structure may be involved (Lee and George 2005; Das et al. 2007; Schreiner 2007).

8.3.4 Defense Responses in Mutualistic Plant/Microbe Interactions

Defense related gene expression has been intensively studied in mycorrhiza. Mutualistic interactions between host and mycorrhizal fungi require a balance between the defense responses generated by these defense related gene expressions of the host plant and the nutrient demand of the fungus. These genes in mycorrhiza are mainly upregulated during early stages of infestation (García-Garrido and Ocampo 2002; Gao et al. 2004; Grünwald et al. 2004) and downregulated as the development of the symbioses progresses (Harrison 2005; Hause and Fester 2005; Paszkowski 2006). Thus, either beneficial fungi do not stimulate extensive defense gene expression over longer periods or they actively downregulate them after an initial activation period (Volpin et al. 1994) presumably during haustoria formation (Harrison 2005). It has been studied that transgenic tobacco plants constitutively expressing pathogenesis related (PR) proteins can be colonized by the arbuscular mycorrhizal fungus *Glomus mosseae* (Vierheilig et al. 1995). Microarray analysis uncovered that defense genes are moderately up-regulated during early phases of the interaction in *Arabidopsis* roots and that their expression declines during later phases. Activation of defense gene expression can even be detected before the two

partners attain physical contact and are responsible to control, at least in part, the intraradical fungal growth (Lambais and Mehdy 1995). The processes determining the functional integration between the symbionts of mycorrhizal associations are being obtained by using approaches based on the analysis of the proteins and gene expressions.

8.3.5 Biocontrol: Plant Protection in the Rhizosphere

Beneficial microorganisms interact with the plant pathogens directly and indirectly both (Benítez et al. 2004; Viterbo et al. 2007; Arabi et al. 2013). The direct confrontation between both parties involves physical contact resulting in normal reactions from both of them, such as synthesis and secretion of metabolites (Bais et al. 2006; Sui et al. 2013). These metabolites can be mainly hydrolytic enzymes, toxic compounds or certain antibiotics. However, use of organic soil amendments for improving the antagonistic activity against pathogen has been reported to induce resistance in the host plant which is an indirect method of interaction (Benítez et al. 2004; Pal and Gardener 2006; Viterbo et al. 2007). The important mechanisms of biocontrol depend upon dynamics and chemical attributes of soil such as temperature and pH. The rhizospheric environment and presence of other microorganisms (Howell 2003) are other contributing factors. Important principle mechanisms of biological control include both direct as well as indirect interactions such as competition, antibiosis, induced resistance, and lysis (Irtwange 2006; Viterbo et al. 2007; Tripathi et al. 2008).

Antibiosis is the inhibition or destruction of microorganism by metabolic compounds known as antibiotics that inhibit the growth of another microorganism (Benítez et al. 2004, Irtwange 2006; Haggag and Mohamed 2007; Viterbo et al. 2007). These antibiotics play a significant role in disease suppression by 'static' or 'cidal' mechanisms (Benítez et al. 2004; Haggag and Mohamed 2007). In synergistic combination with several other cell wall degrading enzymes, these antibiotics produce a strong inhibitory effect on many plant pathogens (Benítez et al. 2004; Woo and Lorito 2007; Vinale et al. 2008).

Limited space and nutrition in the rhizosphere compels the microbes to undergo competition (Lewis et al. 1989; Howell 2003; Benítez et al. 2004; Viterbo et al. 2007). Beneficial microorganisms tend to displacement and removal of the pathogen due to competition between them. They compete with other pathogenic microbes for food essential nutrients in the rhizospheric soil (Chet et al. 1990; Irtwange 2006) and modify the soil dynamics in various ways so that pathogen growth is inhibited (Benítez et al. 2004).

Enzymatic activity from the mycorrhizal fungi holds significant importance in control of pathogens mainly by lytic mechanism (Viterbo et al. 2007). The lytic enzymes such as chitinases, proteases, and β-1,3 glucanases (Whipps 2001) lyse pathogen hyphal cell walls during mycoparasitic activity (El-Katathy et al. 2001; Khetan 2001). Also β-1,3 glucanases and proteases have been reported to cause

degradation of cell walls and mycelia growth and spore germination inhibition which eventually inhibit the growth of pathogenic fungi (Benítez et al. 2004; Lin et al. 2007). They are supposed to deactivate the hydrolytic enzymes produced by pathogens and thus help in the reduction of disease severity (Elad and Kapat 1999).

In response to pathogen infestation most plants develop kind of resistance towards them (Harman et al. 2004). These resistances are induced in adverse situations and based upon type and source of stimulus, can be localized and/or systematic in nature (Pal and Gardener 2006). Mycorrhizal fungi establish interaction induced metabolic changes and involve genes and expressions known as systematic acquired resistance, induced systematic resistance or hypersensitive responses (Handelsman and Stabb 1996; Whipps 2001) in plants which is responsible for increased resistance to various plant pathogens (Benítez et al. 2004; Haggag 2008). Certain metabolites and defense related enzymes such as phenylalanine ammonio-lyase and chalcone synthase have been reported to be involved in the biosynthesis of phytoalexins, chitinases and glucanases (Benítez et al. 2004; Viterbo et al. 2007) which are responsible for pathogen inhibition. Also, certain proteins, peptides, and low molecular- weight compounds produced by mycorrhizae may act as elicitors of plant resistance and show plant defense responses (Benítez et al. 2004; Viterbo et al. 2007).

8.4 Production of Mycorrhizal Fungi as Biofertilizers and Bioprotectants

The criteria for selecting mycorrhizal fungi for their formulation as biofertilizers and bioprotectants depend on details of the local environment, soil conditions, and host plants. The mycorrhizal fungi must be capable of rapid root colonization after inoculation, should efficiently absorb and transfer phosphorus from the soil to the plant root, should be strong enough to persist and form propagules that remain viable and re-establish the symbiotic relationship, and it should increase plant growth (Bagyaraj et al. 2002; Singh and Tilak 2002; Tanu et al. 2006). Increased populations of propagules by mass production can help to effectively manage and exploit these beneficial fungi for potential usage as biofertilizer and bioprotectants (Smith and Zhu 2001; Tiwari et al. 2004; Kapoor et al. 2008).

Mass production of the mycorrhizal inocula has been reported to be achieved by multiplication of selected fungi in roots of susceptible host plants (Naqvi and Mukerji 2000; Yeasmin et al. 2007; Marleen et al. 2011) for their application as spores, or fragments of colonized roots. Spore inocula have been the most resistant and can survive unfavourable environmental conditions for a long period. The spores and hyphae at a larger scale after isolation from the soil rhizosphere were mixed with carrier substrates for application (Gentili and Jumpponen 2006). In view that the spores inocula colonize new root systems more slowly than other preparations, both types of inocula, e.g. spores and fragments of colonized roots

should be combined in commercial products (Marin 2006). Mass production of mycorrhizal fungi has been achieved with several species such as *Acaulospora laevis*, *Glomus. clarum*, *G. etunicatum*, *G. intraradices*, *G. mosseae* (Chandanie et al. 2006), *Gigaspora ramisporophora* and *G. rosea* (Schwartz et al. 2006) but *Glomus intraradices* is the most common inoculum of endomycorrhizae products (Douds et al. 2000; Adholeya et al. 2005; Wu et al. 2005; Schwartz et al. 2006; Akhtar and Siddiqui 2008).

Some steps are essential for development of a commercial fungal biofertilizers once the mass cultivation of the desired organism has been achieved. They include selection, large scale production, carrier selection and preparation, mixing and curing, maintenance of appropriate numbers of inocula, and strong quality control (Malik et al. 2005). Basis of a mycorrhizal formulation to be successful depends upon its economical viability to produce, stable viability and function of the inoculum and the ease for efficient and enhanced dispersal during application. Besides, numerous other factors such as crop species, size and effectiveness of indigenous mycorrhizal fungi populations, fertility of the soil, and cultural practices have also been studied for the sustained effects of these biofertilizer preparations (Adholeya et al. 2005; Hart and Trevors 2005; Tiwari and Adholeya 2005; Gianinazzi et al. 2010). There are various types of mycorrhiza products comprising one or multiple mycorrhizal fungal inocula (Singh et al. 2008) from more than thirty companies involved in their worldwide marketing (Raja 2006; Schwartz et al. 2006).

8.5 Conclusion

The benefits of using mycorrhizal fungi as biofertilizers and bioprotectants include plant growth promotion, enhanced production of bioactive compounds and secondary metabolites, decreased occurrence of plant diseases, pathogen suppression, disease resistance, increased uptake and availability of nutrients from the soil to plants, regulation of hormones and enzymes production for plant growth stimulation. All these are beneficial aspects for increased crop production and sustainable agricultural practices. Exploitation of mycorrhizal fungi as biofertilizers and bioprotectants at commercial level provide more environmental friendly alternative approach than chemicals used as fungicides and fertilizers. The efficiency and success rate of these products can be increased by further research to enhance their stability, shelf life, mechanism of action, mode of action and certain other factors which are useful for the sustainable agricultural practices.

References

Adholeya A, Tiwari P, Singh R (2005) Large scale inoculum production of arbuscular mycorrhizal fungi on root organs and inoculation strategies. In: Declerck S, Strullu DG, Fortin A (eds) *In Vitro* culture of mycorrhizae. Springer, Berlin, pp 315–338

Akhtar SM, Siddiqui ZA (2008) Biocontrol of a root-rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp. and *Pseudomonas straita*. Crop Prot 27:410–417

Ames RN (1987) Mycorrhizosphere morphology and microbiology. In: Sylvia DM, Hung LL, Graham SH (eds) Mycorrhizae in the next deacade. Proc 7th NACOM, Gainesville, FL

Arabi MIE, Ayoubi SKZ, Jawhar M (2013) Mycorrhizal application as a biocontrol agent against common root rot of barley. Res Biotechnol 4:7–12

Arnold WN (1981) Enzymes. In: Arnold WN (ed) Yeast cell envelope: biophysics, biochemistry and ultrastructure II. CRC Press, Boca Raton, FL, pp 1–46

Ashford AE, Jacobson JV (1974) Cytochemical localization of phosphatase in barley aleurone cells: the pathway of gibberellic acid induced enzyme release. Planta 120:81–105

Azcon-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi to the control of soil borne pathogens. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandte K (eds) Mycorrhizal technology in agriculture. Birkhauser, Basel, pp 187–198

Bagyaraj DJ, Mehrotra VS, Suresh CK (2002) Vesicular arbuscular mycorrhizal biofertilizer for tropical forest plants. In: Kannaiyan S (ed) Biotechnology of biofertilizers. Kluwer Academic, Boston, MA, pp 299–311

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

Bakkaus E, Collins RN, Morel JL, Gouget B (2006) Anion exchange liquid chromatography inductively coupled plasma-mass spectrometry of the Co^{2+},Cu^{2+}, Fe^{3+} and Ni^{2+} complexes of mugineic and deoxymugineic acid. J Chromatogr 1129:208–215

Bansal M, Mukerji KG (1994) Efficacy of root litter as a biofertilizer. Biol Fertil Soils 18:228–230

Basha SM (1984) Purification and characterization of an acid phosphatase from peanut (*Arachis hypogaea*) seed. Can J Bot 62:385–391

Baslam M, Pascual I, Sanchez-Díaz M, Erro J, García-Mina J et al (2011) Improvement of nutritional quality of greenhouse-grown lettuce by arbuscular mycorrhizal fungi is conditioned by the source of phosphorus nutrition. J Agric Food Chem 59:11129–11140

Benítez T, Rincón MA, Limón MC, Codón CA (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7:249–260

Bharadwaj DP, Lundquist PO, Alstrom S (2008) Carbon nanomaterial from tea leaves as an anode in lithium secondary batteries. Asian J Exp Sci 22:89–93

Bidartondo MI, Redecker D, Hijrl 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

Blasius D, Feil W, Kottke I, Oberwinkler F (1986) Hartig net formation in fully ensheated ectomycorrhizas. Nordic J Bot 6:837–842

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun 1:4. https://doi.org/10.1038/ncomms1046

Bonfante P, Perotto S (1995) Tansley review No. 82. Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol 130:3–21

Bothe H, Turnau K, Regvar M (2010) The potential role of arbuscular mycorrhizal fungi in protecting endangered plants and habitats. Mycorrhiza. https://doi.org/10.1007/s00572-010-0332-4

Brundrett M (2004) Diversity and classification of mycorrhizal associations. Biol Rev 79:473–495

Burleigh SH, Cavagnaro T, Jakobsen I (2002) Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition. J Exp Bot 53:1593–1601

Buyer JS, Kratzke MG, Sikora LJ (1993) A method for detection of pseudobactin, the siderophore prodaced by a plant-growth-promoting *Psuedomonas* strain, in the barley rhizosphere. Appl Environ Microbiol 59:677–681

Cakmak I, Gülüt KY, Marschner H, Graham RD (1994) Effect of zinc and iron deficiency on phytosiderophore release in wheat genotypes differing in zinc efficiency. J Plant Nutr 17:1–17

Caris C, Hördt W, Hawkins HJ, Römheld V, George E (1998) Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35–39

Casarin V, Plassard C, Hinsinger P, Arvieu JC (2004) Quantification of ectomycorrhizal effects on the bioavailability and mobilization of soil P in the rhizosphere of *Pinus pinaster*. New Phytol 163:177–195

Cavagnaro TR, Gao LL, Smith FA, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytol 151:469–475

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D et al (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282:209–225

Chalot M, Javelle A, Blaudez D, Lambilliote R, Cooke R, Sentenac H, Wipf D, Botton B (2002) An update on nutrient transport processes in ectomycorrhizas. Plant Soil 244:165–175

Chandanie WA, Kubota M, Hyakumachi M (2006) Interactions between plant growth promoting fungi and arbuscular mycorrhizal fungus *Glomus mosseae* and induction of systemic resistance to anthracnose disease in cucumber. Plant Soil 286:209–217

Chet I, Ordentlich A, Shapira R, Oppenheim A (1990) Mechanisms of biocontrol of soil-borne plant pathogens by *Rhizobacteria*. Plant Soil 129:85–92

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

Clark RB, Zeto SK (1996) Mineral acquisition by mycorrhizal maize grown on acid and alkaline soil. Soil Biol Biochem 28:1495–1503

Colla G, Rouphael 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 Soil 44:501–509

Das A, Prasad R, Srivastava A, Giang PH, Bhatnagar K, Varma A (2007) Fungal siderophores: structure, functions and regulation. In: Varma A, Chincholkar SB (eds) Microbial siderophores, Soil biology. Springer, Berlin, pp 1–42

Dhamija SS, Fluri R, Schweingruber ME (1987) Two genes control three alkaline phosphatases in *Schizosaccharomyces pombe*. Curr Genet 11:467–473

Douds DD, Gadkar JV, Adholeya A (2000) Mass production of VAM fungus biofertilizer. In: Mukerji KG, Singh J, Chamola BP (eds) Mycorrhizal biology. Kluwer Academic, New York, pp 197–214

Elad Y, Kapat A (1999) The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. Eur J Plant Pathol 105:177–189

El-Katathy MH, Gudelj M, Robra KH, Elnaghy MA, Gübitz GM (2001) Characterization of a chitinase and an endo-β-1, 3-glucanase from *Trichoderma harzianum* Rifai T24 involved in control of the phytopathogen *Sclerotium rolfsii*. Appl Microbiol Biotechnol 56:137–143

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Ezawa T, Hayatsu M, Saito M (2005) A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. Mol Plant Microbe Interact 18(10):1046–1053

Friberg S (2001) Distribution and diversity of arbuscular mycorrhizal fungi in traditional agriculture on Niger inland delta, Mali, West Africa. CBN:s Skriftserie 3:53–80

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

García-Garrido JM, Ocampo JA (2002) Regulation of the plant defense response in arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386
Gentili F, Jumpponen A (2006) Potential and possible uses of bacterial and fungal biofertilizers. In: Rai MK (ed) Handbook of microbial biofertilizers. Food Products Press, New York, pp 1–28
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
Giri B, Mukerji KG (2004) Mycorrhizal inoculant alleviates 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, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmuller R, Varma A (2005) Mycorrhizosphere: strategies and functions. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Heidelberg, pp 213–252
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, Heidelberg, pp 3–28
Gosling P, Hodge A, Goodlass G, Bending GC (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35
Grünwald U, Nyamsuren O, Tamasloukht M, Lapopin L, Becker A, Mann P, Gianinazzi-Pearson-V, Krajinski F, Franken P (2004) Identification of mycorrhiza-regulated genes with arbuscule development-related expression profile. Plant Mol Biol 55:553–566
Haggag WM (2008) Biotechnological aspects of plant resistant for fungal diseases management. Am Eurasian J Sustain Agric 2:1–18
Haggag WM, Mohamed HAA (2007) Biotechnological aspects of microorganisms used in plant biological control. Am Eurasian J Sustain Agric 1:7–12
Handelsman J, Stabb VE (1996) Biocontrol of soilborne plant pathogens. Plant Cell 8:1855–1869
Harley JL, Smith SE (1983) Mycorrhizal symbiosis, 1st edn. Academic Press, London
Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species opportunistic, a virulent plant symbionts. Nat Rev Microbiol 2:43–56
Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42
Hart MM, Trevors JT (2005) Microbe management: application of mycorrhyzal fungi in sustainable agriculture. Front Ecol Environ 3:533–539
Hasegawa Y, Lynn KR, Brockbank WJ (1976) Isolation and partial characterization of cytoplasmic and wall-bound acid phosphatase from wheat roots. Can J Bot 54:1163–1169
Hause B, Fester T (2005) Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta 221:184–196
Howell RC (2003) Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87:4–10
Irtwange VS (2006) Application of biological control agents in pre- and postharvest operations. Agricultural Engineering International: the CIGR Ejournal Invited Overview, vol 3, pp 1–12
Jakobsen I, Rosendahl I (1991) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83
Javaid A (2009) Arbuscular mycorrhizal mediated nutrition in plants. J Plant Nutr 32:1595–1618
Kapoor R, Bhatnagar AK (2007) Attenuation of cadmium toxicity in mycorrhizal Celery (*Apium graveolens* L.) World J Microbiol Biotechnol 20:1083–1089
Kapoor R, Sharma D, Bhatnagar AK (2008) Arbuscular mycorrhizae in micropropagation systems and their potential applications. Sci Hortic 116:227–239
Khan MS, Zaidi A, Musarrat J (2009) Microbial strategies for crop improvement. Springer, Berlin
Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734
Khetan SK (2001) Microbial pest control. Marcel Dekker, New York, p 300

Lambais MR, Mehdy MC (1995) Differential expression of defense-related genes in arbuscular mycorrhiza. Can J Bot 73:S533–S540

Lee YJ, George E (2005) Contributions of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. Plant Soil 278:361–370

Lewis K, Whipps JM, Cooke RC (1989) Mechanisms of biological disease control with special reference to the case study of *Pytium oligandrum* as an antagonist. In: Whipps JM, Lumsden RD (eds) Biotechnology of fungi for improving plant growth. Cambridge University Press, Cambridge, pp 191–217

Leyval C, Reid CPP (1991) Utilization of microbial siderophores by mycorrhizal and non-mycorrhizal pine roots. New Phytol 119:93–98

Li AR, Guan KY, Stonor R, Smith SE, Smith FA (2013) Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic *Pedicularis* species: do the fungal partners matter at low colonization levels? Ann Bot 112:1089–1098

Lin C, Yang J, Sun H, Huang X, Wang R, Zhang KQ (2007) Purification and characterization of a β-1, 3-glucanase from the novel mycoparasite *Periconia byssoides*. Biotechnol Lett 29:617–622

Linderman RG (1988) Mycorrhizal interactions with rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78:366–371

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) growing in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Liu Q, Loganathan P, Hedley MJ, Grace LJ (2008) Effect of mycorrhizal inoculation on rhizosphere properties, phosphorus uptake and growth of pine seedlings treated with and without a phosphate rock fertilizer. J Plant Nutr 31:137–156

Malik KA, Hafeez FY, Mirza MS, Hameed S, Rasul G, Bilal R (2005) Rhizospheric plant – microbe interactions for sustainable agriculture. In: Wang YP, Lin M, Tian ZX, Elmerich C, Newton WE (eds) Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Berlin, pp 257–260

Marin M (2006) Arbuscular mycorrhizal inoculation in nursery practice. In: Rai MK (ed) Handbook of microbial biofertilizers. Food Products Press, New York, pp 289–324

Marleen I, Sylvie C, Stéphane D (2011) Methods for large scale production of AM fungi: past, present and future. Mycorrhiza 21:1–16

Marler MJ, Zabinski CA, Callaway RM (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. Ecology 80:1180–1186

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, Barea JM, Azcon R (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

Meddad-Hamza A, Beddiar A, Gollotte A, Lemoine MC, Kuszala C, Gianinazzi S (2010) Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. Afr J Biotechnol 9:1159–1167

Milagres AMF, Machuca A, Napoleao D (1999) Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. J Microbiol Methods 37:1–6

Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol 12:563–569

Mukerji KG, Chamola BP, Singh J (eds) (2000) Mycorrhizal biology. Kluwer Academic, New York

Naqvi NS, Mukerji KG (2000) Mycorrhizal technology in plant micropropagation system. In: Mukerji KG, Chamola BP, Singh J (eds) Mycorrhizal biology. Kluwer Academic, New York, pp 217–233

Nunes JLD, de Souza PVD, Marodin GAB, Fachinello JC (2010) Effect of arbuscular mycorrhizal fungi and indole butyric acid interaction on vegetative growth of 'Aldrighi' peach rootstock seedlings. Cienc Agrotecnol 34:80–86

Pal K, Gardener BM (2006) Biological control of plant pathogens. Plant Health Instructor 2:1117–1142. https://doi.org/10.1094/PHI-A-2006-1117-02. APSnet: 1–25

Paszkowski U (2006) Mutualism and parasitism: the yin and yang of plant symbioses. Curr Opin Plant Biol 9:364–370

Pedregosa AM, Pinto F, Monistrol IF, Laborda F (1991) Regulation of acid and alkaline phosphatases of *Cladosporium cucumarinum* by inorganic phosphate. Mycol Res 95:720–724

Peterson RL, Massicotte HB (2004) Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. Can J Bot 82:1074–1088

Peyronel B, Fassi B, Fontana A, Trappe JM (1969) Terminology of mycorrhizae. Mycologia 61:410–411

Plassard C, Dell B (2010) Phosphorus nutrition of mycorrhizal trees. Tree Physiol 30:1129–1139

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, Berlin, pp 1–7

Raina S, Chamola BP, Mukerji KG (2000) Evolution of mycorrhiza. In: Mukerji KG, Singh J, Chamola BP (eds) Mycorrhizal biology. Kluwer Academic/Plenum Plublishers, New York, pp 1–25

Raja P (2006) Status of endomycorrhizal (AMF) biofertilizer in the global market. In: Rai MK (ed) Handbook of microbial biofertilizers. Food Products Press, New York, pp 395–416

Rillig MC, Mardatin NF, Leifheit EF, Antunes PM (2010) Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates. Soil Biol Biochem 42:1189–1191

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45

Romheld V (1987) Different strategies for iron acquisition in higher plants. Physiol Plant 90:231–234

Rooney DC, Prosser JI, Bending GD, Baggs EM, Killham K, Hodge A (2011) Effect of arbuscular mycorrhizal colonisation on the growth and phosphorus nutrition of *Populus euramericana* c.v. Ghoy. Biomass Bioenerg 35:4605–4612

Ruiz-Lozano JM, Aroca R (2010) Host response to osmotic stresses: stomatal behaviour and water use efficiency of arbuscular mycorrhizal plants. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 239–256

Scheidegger C, Brunner I (1993) Freeze-fracturing for low-temperature scanning electron microscopy of Hartig net in synthesized *Picea abies* – *Hebeloma crustuliniforme* and *Tricholoma vaccinum* ectomycorrhizas. New Phytol 123:123–132

Schliemann W, Ammer C, Strack D (2008) Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochem 69:112–146

Schreiner PR (2007) Effect of native and non native 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

Schreiner R, Mighara KL, McDaniel II, Bethlenfalvay GJ (1997) Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188:199–209

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol Res 105:1421–1423

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

Selvakumar G, Thamizhiniyan P (2011) The effect of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on the growth and yield of chilli (*Capsicum annuum* L.) under salinity stress. World Appl Sci J 14:1209–1214

Sharda JN, Koide RT (2010) Exploring the role of root anatomy in P-mediated control of colonization by arbuscular mycorrhizal fungi. Bot 88:165–173
Sharma MP (2001) Biodiversity and role of potential isolates of VA-mycorrhizae in various plant species of economic value. PhD thesis, Jiwaji University, Gwalior, Central for Mycorrhizal research, TATA Energy Research Institute, New Delhi
Sheng M, Tang M, Chen H, Yang BW, Zhang FF, Huang YH (2009) Influence of arbuscular mycorrhizae on the root system of maize plants under salt stress. Can J Microbiol 55:879–886
Shi AD, Li Q, Huang J, Yuan L (2013) Influence of arbuscular mycorrhizal fungi on growth, mineral nutrition and chlorogenic acid content of *Lonicera confusa* seedlings under field conditions. Pedosphere 23:333–339
Shinde SK, Shinde BP, Patale SW (2013) The alleviation of salt stress by the activity of AM fungi in growth and productivity of onion (*Allium cepa* l.) plant. Int J Life Sci Pharma Res 3:11–15
Silvia GA, Trufem SFB, Saggin JOJ, Maia LC (2005) Arbuscular mycorrhizal fungi in a semi-arid copper mining area in Brazil. Mycorrhiza 15:47–53
Singh PK (2012) Role of glomalin related soil protein produced by arbuscular mycorrhizal fungi: a review. Agric Sci Res J 2:119–125
Singh G, Tilak KVBR (2002) Vesicular arbuscular mycorrhizal as bioinoculant. In: Kannaiyan S (ed) Biotechnology of biofertilizers. Kluwer Academic, Boston, MA, pp 312–322
Singh S, Pandey A, Palni LMS (2008) Screening of arbuscular mycorrhizal fungal consortia developed from the rhizospheres of natural and cultivated tea plants for growth promotion in tea (*Camellia sinensis* (L.) O. Kuntze). Pedobiologia 52:119–125
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London
Smith FA, Smith SE (1997) Tansley review No. 96. Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. New Phytol 137:373–388
Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104:1–13
Smith SE, Zhu YG (2001) Application of arbuscular mycorrhizal fungi: potentials and challenges. In: Stephen BP, Hyde KD (eds) Bio-exploitation of filamentous fungi, Fungal diversity research series 6, pp 291–308
Smith FW, Rae AL, Hawkesford MJ (2000) Molecular mechanisms of phosphate and sulphate transport in plants. Biochim Biophys Acta 1465:236–245
Smits MM, Bonneville S, Haward S, Leake JR (2008) Ectomycorrhizal weathering, a matter of scale. Mineral Mag 72:131–134
Sui XL, Li AR, Chen Y, Guan KY, Zhuo L, Liu YY (2013) Arbuscular mycorrhizal fungi: potential biocontrol agents against the damaging root hemiparasite Pedicularis kansuensis? Mycorrhiza. http://www.ncbi.nlm.nih.gov/pubmed/24077881 [Epub ahead of print]
Takeda N, Kistner C, Kosuta S, Winzer T, Pitzschke A, Groth M et al (2007) Proteases in plant root symbiosis. Phytochem 68:111–121
Tanu, Prakash A, Adholeya A (2006) Potential of arbuscular mycorrhizae in organic farming system. In: Rai MK (ed) Handbook of microbial biofertilizers. Food Products Press, New York, pp 223–239
Tao G, Liu ZY, Hyde KD, Yu ZN (2008) Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (*Orchidaceae*). Fungal Divers 33:101–122
Tibbett M, Sanders FE (2002) Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. Ann Bot 89:783–789
Tiwari P, Adholeya A (2005) Root organ culture of arbuscular mycorrhizal fungi: step towards reaching sustainable agriculture. Mycorrhiza News 17:15–17
Tiwari P, Adholeya A, Prakash A (2004) Commercialization of arbuscular mycorrhizal biofertilizers. In: Arora DK (ed) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, New York, pp 195–203
Trappe JM (2005) A.B. Frank and mycorrhizae: the challenge to evolutionary and ecologic theory. Mycorrhiza 15:277–281

Tripathi S, Kamal S, Sherameti I, Oelmuller R, Varma A (2008) Mycorrhizal fungi and other root endophytes as biocontrol agents against root pathogens. In: Varma A, Hock B (eds) Mycorrhizae, 3rd edn. Springer, Heidelberg, pp 281–306

van Aarle IM, Plassard C (2010) Spatial distribution of phosphatase activity associated with ectomycorrhizal plants is related with soil type. Soil Biol Biochem 42:324–330

Varma A, Hock B (eds) (1998) Mycorrhiza: structure, function, molecular biology, and biotechnology. Springer, New York

Vierheilig H, Alt M, Lange J, Gut-Rella M, Wiemken A, Boller T (1995) Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. Appl Environ Microbiol 61:3031–3034

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma* plant pathogen interactions. Soil Biol Biochem 40:1–10

Viterbo A, Inbar J, Hadar Y, Chet I (2007) Plant disease biocontrol and induced resistance via fungal mycoparasites. In: Kubicek CP, Druzhinina IS (eds) Environmental and microbial relationships, The mycota IV, 2nd edn. Springer, Berlin, pp 127–146

Vivas A, Azcon R, Biro B, Barea JM, Ruiz-Lozano JM (2003) Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pratense* L. under lead toxicity. Can J Microbiol 49:577–588

Volpin H, Elkind Y, Okon Y, Kapulnik Y (1994) A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defense response in alfalfa roots. Plant Physiol 104:683–689

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Willis A, Rodriguesb BF, Harrisa PJC (2013) The ecology of arbuscular mycorrhizal fungi. Crit Rev Plant Sci 32:1–20

Woo LS, Lorito M (2007) Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. In: Vurro M, Gressel J (eds) Novel biotechnologies for biocontrol agent enhancement and management. Springer, Dordrecht, pp 107–130

Wu QS, Xia RX (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425

Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166

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

Yeasmin T, Zaman P, Rahman A, Absar N, Khanum NS (2007) Arbuscular mycorrhizal fungus inoculum production in rice plants. Afr J Agric Res 2:463–467

Zhang F, Römheld V, Marschner H (1991) Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron deficient wheat. Soil Sci Plant Nutr 37:671–678

Zhang YF, Wang P, Yang YF, Bi Q, Tian SY, Shi XW (2011) Arbuscular mycorrhizal fungi improve reestablishment of *Leymus chinensis* in bare saline–alkaline soil: Implication on vegetation restoration of extremely degraded land. J Arid Environ 75:773–778

Zhu GS, Yu ZN, Gui Y, Liu ZY (2008) A novel technique for isolating orchid mycorrhizal fungi. Fungal Divers 33:123–137

Chapter 9
Management of Fungal Pathogens by Mycorrhiza

Ram Prasad, Nomita Gupta, Urvashi Satti, Shanquan Wang, Ahmed IS Ahmed, and Ajit Varma

Abstract Mycorrhiza is a symbiotic association between a fungus and host plant, it has been reported to be mutually beneficial for both the partners. 90% terrestrial plant species able to colonize by mycorrhizal fungal species ranging from flowering to non-flowering plants, 10% terrestrial plants do not form such type of association. Arbuscular mycorrhizae (AM) are the symbiotic fungi that predominate in the soils and roots of important crop plants for human mankind. The AM is the major type that abundant and form beneficial symbiosis with terrestrial ecosystems and crop production systems. The AM might complete its partial life cycle in host system. The negative-antagonistic interaction of AM with various soil borne plant pathogenic fungi is the explanation for their potential use as bio-control agents. Many researchers have experimentally observed antagonistic effects of AM against some fungal pathogens. This chapter will highlighting on arbuscular mycorrhiza, types of mycorrhiza, the interaction between mycorrhizae and plant pathogens, the role of mycorrhizae in activation of plant defence mechanisms, and effect of some nanoparticles types on mycorrhizae and pathogenic fungi.

R. Prasad (✉) • N. Gupta • U. Satti • A. Varma
Amity Institute of Microbial Technology, Amity University, E-3, Fourth Floor, Noida, Uttar Pradesh 201313, India
e-mail: rprasad@amity.edu; rpjnu2001@gmail.com

S. Wang
School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou 510006, China

A.I. Ahmed
Amity Institute of Microbial Technology, Amity University, E-3, Fourth Floor, Noida, Uttar Pradesh 201313, India

Plant Pathology Unit, Plant Protection Department, Desert Research Center, Cairo, Egypt

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_9

9.1 Introduction

Fungi are the cosmopolitans; they can survive in different habitats. From two common groups of fungus, there is one beneficial group like mycorrhizae and another are harmful like pathogenic fungi (Perotto et al. 2013). Almost all phytopathogenic fungi spent half of their life cycle in host plant and remaining life cycle outside the host either in soil or in plant debris. Plant diseases arise by the attack of pathogenic fungi, bacteria, viruses, insects or parasitic plants. The diseases can be manifested by decayed roots, shrivelled or loss of fruit, wilting leaves, necrotic lesions on stem or the cankers formation. The survival and performance of most of the phytopathogenic fungi depend upon the prevailing conditions of temperature, moisture and presence of water in their environment (Lin et al. 1991).

Beneficial fungi like mycorrhizae (fungi symbiotic association with the higher plants root) can be used as control agents of pathogenic fungi. There are various types of mycorrhizae present across the globe but the most abundant in arbuscular mycorrhizae (AM) which are commonly seen in about 90% of higher plants. Most of the investigation have been reported that AM fungi have potential to enhance mycorrhizal colonization and lead to plant growth and yield escalation (Bagyaraj 2014). Metal nanoparticles affect the AM resulting in increase of metallic stress on plants, some metallic nanoparticles increase the AM growth and some are very toxic for them.

9.2 Types of Mycorrhizae

Mycorrhiza term *w*as first pointed by a German scientist Frank A.B. in 1885, and was originated from the "*mycos"* Greek word that meaning "fungus" while *rhiza* meaning 'root'. Mycorrhiza is a symbiotic describes the relationship between the soil fungi and the structure which is formed from the complex of both (Muchovej 2001). Most of terrestrial plants root system forms complex with fungi. These are the ubiquitous symbioses, known as mycorrhizas, and they role as conduits for the energy flow and matter between soil and plants (Cardon and Whitbeck 2007). The association is mutualistic which means both organisms get the benefits from each of them. The fungi receive the required carbohydrates (sugars) and required growth factors from the plant for its normal metabolism, while the plant receives benefits, like increased the area of surface for absorption of nutrient. The mycorrhizal association which is involved in the absorption of nutrients from soil is found between hyphal fungi and the gametophytes underground organs of many pteridophytes and bryophytes, and also the roots of plants as well as the sporophytes of most types of pteridophytes. The common two mycorrhiza types are ectomycorrhiza and endomycorrhiza, where they differ considerably in their structure and physiological relationships with symbionts (Mohammadi et al. 2011).

The "mycorrhizosphere" term describes properties which are unique to the rhizosphere surrounding and influenced by mycorrhizae (Lin et al. 1991; Giri et al. 2005). Mycorrhizal fungi stimulate plants to root biomass reducing and simultaneously expanding uptake of nutrient capacity that is done by extending far of beyond surfaces of root and then proliferating in pores of soil that are not enough for root hairs to enter (Mohammadi et al. 2011). The networks of mycorrhizal fungi lead to connect the plant root systems for broad areas. These fungi frequently comprise the largest portion of soil microbial biomass (Mohammadi 2011). Mainly mycorrhiza could be divided to ectomycorrhiza and endomycorrhiza. In ectomycorrhiza hyphae do not penetrate the cells individually within the root and endomycorrhiza which the fungal hyphae penetrate it through the cell wall and membrane. The principle structural features of the main types of mycorrhiza showed in (Fig. 9.1).

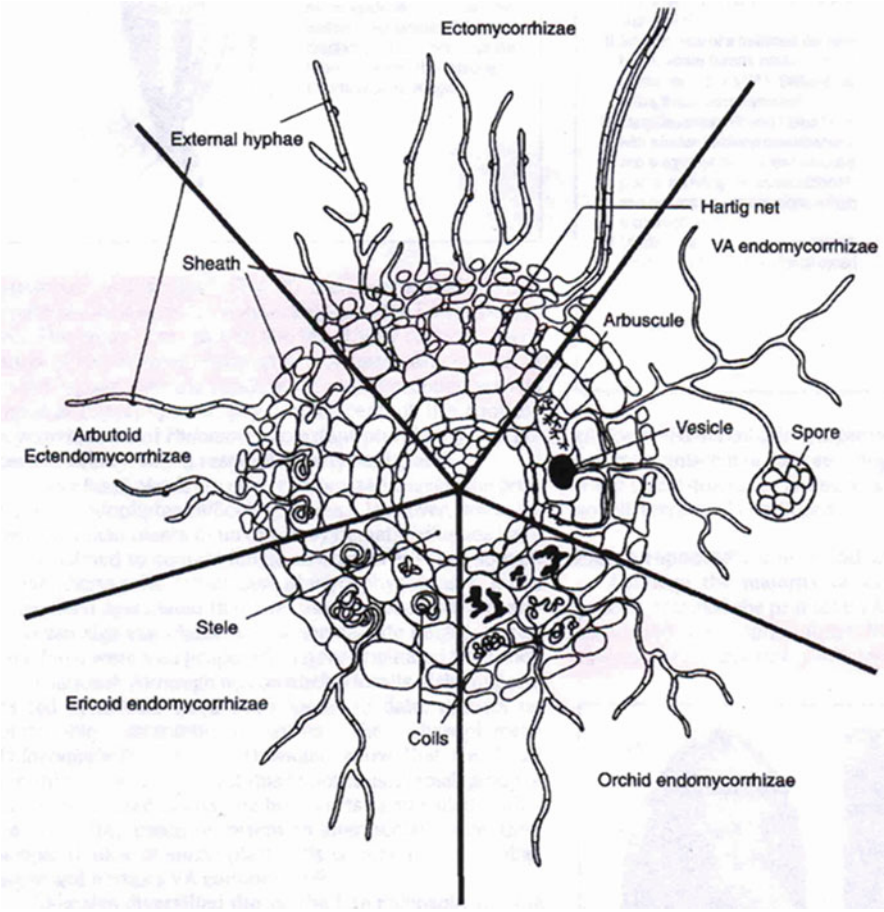


Fig. 9.1 The principle structural features of the main types of mycorrhiza (Selosse and Le Tacon 1998) with permission from Elsevier

9.2.1 Ectomycorrhiza

Ectomycorrhiza are characterized by fungal hyphae sheath surrounds the root of host and is pointed as mantle. This mantle covers the root completely leading to changes in its color and morphology. They have different types of rhizomorph, cystidia etc. projecting out. Fungal hyphae enter inside the root and arranged intercellularly surrounding the cortical cells forming a net like structure, called hartigs net. Both of tertiary and secondary roots then infection root hair production becomes reduced and root gets coated with hyphae. Hyphae grow in between the cells either enzymatically or mechanically. Plants belonging to the family fragaceae, betulaceae, rosaceae, salicaceae etc form ectomycorrhizae. The ectomycorrhizal fungi belong to the classes basidiomycetes and ascomycetes. They are not established in woody shrubs and trees having short roots that do not possesses secondary growth. However they are reported in herbaceous annuals and perennials (Muchovej 2001; Cumming et al. 2015).

9.2.2 Endomycorrhiza

Endomycorrhizae of root forms both inter and intra-cellular hyphae. When hyphae enter in cortical cells it forms various types of fungal structures commonly vesicle, arbuscles and coils are common. Vesicles are actually balloons like structure act as storage organ while arbuscles are bush like hyphae structure. They were known as the arbuscular mycorrhizae (AM), are characterized by arbuscules or vesicles in the plant root cells. Some of these types do not have vesicle, so a common term arbuscular mycorrhizae is mainly used (Abbott and Robson 1981). They are the commonest types of mycorrhizae, which develop in the roots of a wide variety of host plants. AM are considered as obligate biotrophs. They cannot grow and reproduce in the absence of a host pants. These fungi belong to the class zygomycetes and order glomales. AM are beneficial in tropical acidic and infertile soils. Mycorrhizal inoculants are having tremendous value because of their ability to improve the efficiency and sustainability of crop yields in areas of low soil fertility. AM is widely present worldwide and with different groups of host plants. They can be tolerant for wide range of ecological stresses (pH, temperature, moisture, rainfall etc.). AM fungi have a wide host range but they show host preferences. The preferential association of AM with certain hosts shows plant growth stimulation (Moore et al. 2011). Their nutritional requirements may not very specific so that they have wide host range. However, like other biotrophic associations, mycorrhizal infections also show compatibility. They differ in their physiological interaction with different plants, leading to variation in their effect on plant growth. This leads to the introduction of the term efficient or effective strains. The efficient strains infect and colonize roots rapidly as compared to effective strains (Mohammadi et al. 2011).

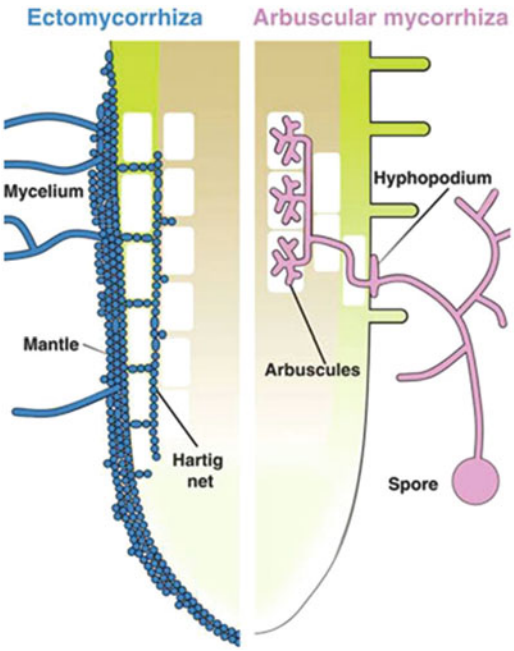


Fig. 9.2 Schematic showing the difference between ectomycorrhizae and endomycorrhizae colonization of plant roots. © Nature Publishing Group. Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nature communications (Bonfante and Genre 2010)

9.2.3 Ectendomycorrhiza

These types of mycorrhizal types are similar to ectomycorrhizae but there is intercellular penetration in between cells. The mantle was covered with mucilage secreted by less developed roots. This kind of mycorrhizae shares the features of both endo and ecto-mycorrhizae commonly found in *Pinus* and *Picea* in addition to some angiosperm. The differences between ectomycorrhizae and endomycorrhizae colonization of plant roots showed in (Fig. 9.2).

9.2.4 Arbutoid Mycorrhiza

These type of fungi found in Ericales basically in genera *Arbutus* and *Pyrola*. The host plant is mostly woody shrub and tree. When the cortical cells have been penetrates by the fungus where it forms extensive coils of hyphae. Many of fungal symbionts which form symbiosis in these plant also form mycorrhizae with conifers, it has been reported that a transition between endo and ecto mycorrhizae exist in the arbutoid type of mycorrhizae accounting for the term ectendomycorrhizae sometimes applied to this phenomenon. The root system of Arbutoideae, like mycorrhizal trees, is heterorhozic and is almost differentiated into long and short roots. The short roots being converted into mycorrhizae with well-defined sheath

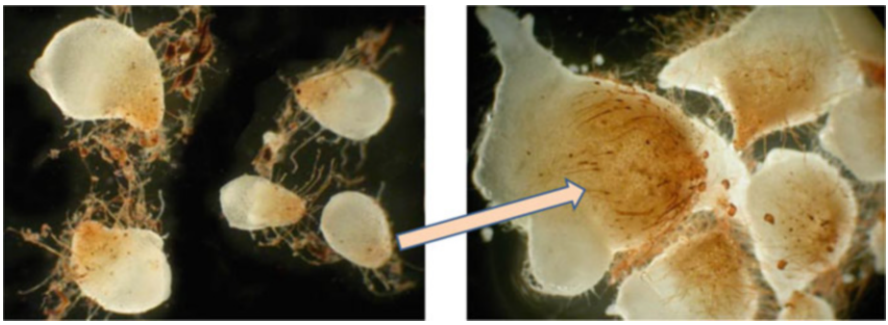


Fig. 9.3 Seedlings of *Rhizanthella gardneri* germinated by a mycorrhizal fungus linked to ECM roots of a shrub (*Melaleuca* sp.). These subterranean seedlings are 2–10 mm long with a zone of brown hyphal coils clearly visible at their base (Moore et al. 2011)

and a hartig net as in Pinus. They may also have a sparse weft of hyphae on the surface of root. The short root covered with the weft of hyphae, the thickness of this varies depending upon the species and root. The Hartig net is formed only in one tier of the cortex and the cell penetrated by hyphae which form coils. These coils become enclosed by interfacial matrix and host plasmalemma (He et al. 2003). Later the fungus and the host cell degenerate. *Cortinarius zakii* is one which forms mycorrhiza in *Arbutus menziesii*. For example, Seedlings of *Rhizanthella gardneri* germinated by a mycorrhizal fungus linked to ectomycorrhizae (ECM) roots of a shrub (*Melaleuca* sp.) is illustrated in (Fig. 9.3).

9.2.5 Ericoid Mycorrhiza

Plants belongs to Ericaceae, Eonpetraceae involve in these mycorrhizal associations. Ericoid mycorrhizae find throughout the fine root system. Several genera such as *Epachris, Leuopogn, Monotoa, Rhododendron* etc. developed ericoid mycorrhizae (Dressier 1993). They have usually fine roots on which fungus established out of corticle cells forming dense intercellular cells. Fungal partner are basidiomycetes or ascomycetes as for example *Pezizella* sp. and *Claveria* sp. This is actually an endomycorrhizal type. The fungi are slow-growing, septate and mostly sterile. They are mostly culturable. Both *Clavaria vermiculata* (Basidiomycotina) and *Pezizella ericae* (Ascomycotina) have been isolated from *Rhododendrons*. During this form the rootlets of the plants are covered by very loose, sparse, dark, septate hyphae that penetrate the cortex forming intercellular hyphal coils (Freudenstein and Doyle 1994). After 3–4 weeks the coils degenerate like arbuscles of AM. Most of the members of ericaceae grow in acidic soil with less amount of phosphorous and nitrogen. The fungus improves the mineral uptake and nutrition of the host, especially phosphorous and nitrogen. Many mycotrophs of Ericaceae showed high resistance to metals like zinc and copper. The mycorrhizal plants also showed high tolerance to these metals, which is totally absent in non-infected plants (Geerinck 1992).

9.2.6 *Orchidoid Mycorrhiza*

Orchidoid mycorrhiza is the association between orchid root and fungi. In orchid the term "mycorrhiza" is used in a broader sense to describe mutualistic plant fungus associations that can be established at different moments in the plant life cycle. Here the fungi colonize either the embryo of the minute orchid seeds or the roots of the chlorophyllous, achlorophyllous orchid species and protocorn. They penetrate the host cells and form intercellular hyphal network. In nature orchids germinate only with infected endomycorrhizal fungi that subsequently colonize the plants, the most common genus *Rhizoctonia* sp. with perfect state *Ceratobasidium*, *Sebacina* occurring in Basidiomycetes and Ascomycetes. The association in pre seedling stage is important in establishment of orchids. The fungi enter in the parenchyma and form coiled structures which looks like arbuscles of AM and digested in cell within short time (Dressier 1993).

9.2.7 *Monotropoid Mycorrhiza*

The achlorophyllous plants which belong the family Monotropaceae such as *Monotropa hypopitys*, develops monotropoid mycorrhizae. The plant partners completely depend upon the fungal mycorrhizae for carbon and energy. The root system of *Monotropa* has main roots bearing tertiary and secondary branches. The roots are enclosed in a sheath of fungi, which is connected with Hartig net involving the outermost layer of host cells. The roots of *Monotropa hypopitys* form a ball like structure throughout which fungal mycelium remifies enclosing the mycorrhizal roots of neighbouring green plants (Smith and Read 2008). The root ball is the survival organ of *Monotropa* sp. During winter and after return of favorable conditions it gives rise to flowering shoots with root growth, sheath and Hartig net. A peg like haustoria from the hyphae push into the epidermal and cortical cells (Yang and Pfister 2006). As the peg grows, glycogen disappears from fungal sheath. These carbohydrates may be employed for another branching formation of the wall of the peg which now grows into the cell. These are similar to transfer the cells and in these transfer regions rough endoplasmic reticulum and mitochondria are plenty. The peg when matures, the tip burst and the contents are released into the sac enclosed by plasmalemma. During this time hyphae from fungal sheath invade and colonize the corticle cells. The functional and structural development of monotropoid mycorrhiza change with seasonal development of the host plants (Harley and Smith 1983).

9.3 Interaction Between Mycorrhizae and Fungal Phytopathogens

Arbuscular mycorrhiza (AM) is common type of mycorrhizae that has ability to inhibit fungal plant pathogens (Artursson et al. 2006). Biological control exploits the antagonistic interactions that reduce the damage caused by fungal pathogen in plants. Fusarium head blight (FHB) is one of disastrous diseases of wheat (*Triticum aestivum)* worldwide, resulted in reduction of grain quality and yield (Parry et al. 1995; Windels 2000). Different species of fungi have been tested for their potential to reduce the inoculums of *Fusarium* pathogens, mainly by reduction in biomass of colonized *Fusarium*. *Tricoderma* is one of the fungal species lives in symbiotic relation with plants reported to have antagonistic abilities against *Fusarium.* It has been confirmed by experiments under field conditions that *Tricoderma* can reduce FHB up to 50% (Perotto et al. 2013). *Gibberella fujikuroi* is the causal agent of bakanae disease in rice especially in seedling stage and overloading with the gibberellins as its own metabolic product. The endophytic fungi *Trichoderma saturnisporum* has maximum inhibitory activity against it (Perotto et al. 2013). Some Gram positive bacteria were isolated from mycorrhizosphere of *Sorgham bicolor* have antagonistic activity towards soil borne fungal pathogens and also stimulates the mycorrhization. There are many reports regarding the potential use of AM fungi as biological control agents against soil borne diseases. The discovery of a *Paenibacillus* strain that is used as a biological control agent against some diseases of soil borne fungi and improving the formation of AM opens the possibility of using dual bacterial-fungal inoculation to ensure the high-value plant production in systems compatible with the environment (Budi et al. 1999). AM can be reduced the variety of pathogenic fungi in roots by either interacting direct and indirect mechanism. These have been proposed based on observations of negative correlations in between the abundance of AM fungal structures and pathogenic microorganisms in soil, roots and in growth medium (St-Arnaud et al. 1995). Arbuscular endomycorrhiza, hyphae from a germinating spore infect a root hair and can grow within the root between root cortical cells and also penetrate the individual cells, forming arbuscules shown in (Fig. 9.4).

AM fungi in multi-species assemblies vary in competitive ability, but the total level of colonization generally does not exceed that of the most abundant fungus when grown individually (Abbott and Robson 1981; White 1984; Jansa et al. 2008). *Glomus fasciculatus* and *Glomus constrictus*, when inoculated in combination, did not increase the frequency of AM fungal root colonization or reduce symptoms of *Phytophthora parasitica* on citrus as compared to when each were inoculated in isolation (Davis and Menge 1981). In another study it has been reported that inoculation with a multi-species AM fungal assemblage from a field soil increased the intensity (and, to a lesser extent, the frequency) of AM fungal colonization of date palm roots, when compared with *Glomus monosporus, Glomus clarum*, or *Glomus deserticola* in isolation, but this did not result in enhanced amelioration of the negative effects of *F. oxysporum* f. sp. *albedinis* on plant growth (Jaiti et al.

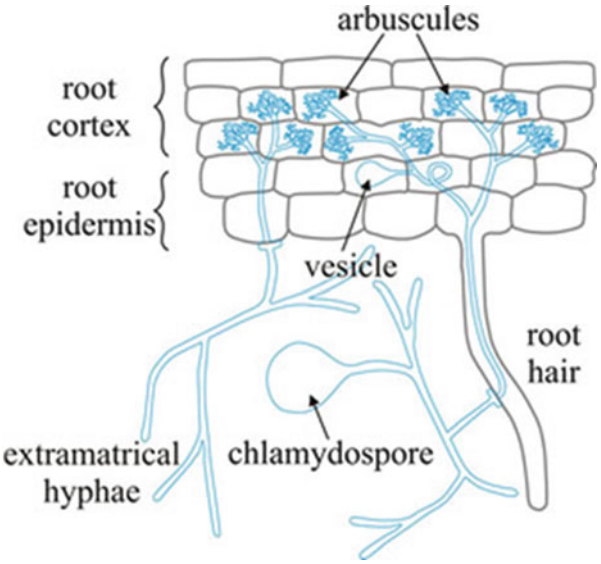


Fig. 9.4 Arbuscular endomycorrhiza: Hyphae from a germinating spore infect a root hair and can grow within the root between root cortical cells and also penetrate individual cells, forming arbuscules (Moore et al. 2011)

2007). Some previous studies suggested that competition between AM and pathogenic fungi were occurred for resources and occupation of space within the root system. The majority of variations in root colonization by AM fungi were explained by the divergence of the two most species-rich fungal clades: the extensively colonizing Glomerales and the poorly colonizing Diversisporales (Powell et al. 2009; Hart and Reader 2002). Surveys on the ability of various AM fungus to reduce the abundance of *F. oxysporum* and/or a *Pythium* sp. in host root systems also suggest, variation in this trait is largely constrained to this divergence, with low levels of pathogen abundance in root systems inoculated with various *Glomus* species relative to those inoculated with various *Gigaspora*, *Scutellospora*, and *Acaulospora* species (Powell et al. 2009; Sikes et al. 2009). Some reports suggested that variation in traits other than the extent of root colonization may be a focus for future surveys. AM fungal colonization influences root architecture of the host plant, mostly by causing a more profusely branched root system (Price et al. 1989; Yano et al. 1996; Paszkowski et al. 2002; Olah et al. 2005; Gutjahr et al. 2009). For several AM species interactions between changes in root system and protection of plant root from pathogen attack are established.

Matsubara et al. (1995) reported that eggplants colonized by *Glomus etunicatum* or *Gigaspora margarita* showed higher lignin concentrations in first and second order roots as compared to non-mycorrhizal plants, when *Verticillium dahliae* was present. These AM fungi caused the plant to produce of the third order roots. *G. mosseae* induced higher branching of the root system but decreased the root branching of tomato under high phosphorous conditions if the plant was attacked by *Phytophthora nicotianae* (Trotta et al. 1996). Newsham et al. (1995) proposed that an abundant of lateral root tips and developing meristems make highly branched root systems more susceptible for pathogen attack, resulting in an increasing

demand for AM fungi for their protection. Norman supported this hypothesis as they compared plants with inherently highly branched root systems and found that mycorrhizal plants had fewer necrosis compared to non-mycorrhizal plants (Norman et al. 1996). If mycorrhizal fungi frequently cause increased branching of the roots, but increased branching in itself leads to higher susceptibility to root pathogen attack; AM fungi must confer protection through additional mechanisms. It is difficult to examine the effects of multiple species of AM fungi for this mechanism.

9.3.1 Activation of Plant Defense Mechanisms

There is establishment between the host plant and AM fungi even before physical contact resulting in specific shifts in the host's gene regulation (Genre et al. 2009; Oldroyd et al. 2009) with consequent production of specific multifunctional compounds (involved in transduction pathways and capable to confer disease resistance) (Pozo and Azcón-Aguilar 2007; Liu et al. 2007; Van Wees et al. 2008). For plants it is an expensive process to constitutively express the defense mechanism rather than AM fungal establishment which enhances the plant ability to activate defense mechanism more efficiently when attacked by fungal pathogen. Such induced pathogen protection may be either systemic within the plants or through root exudation (Lioussanne et al. 2008). Pozo et al. (2002) has reported that *G. mosseae* is very effective in reducing symptoms in *P. parasitica* through the induction of hydrolytic enzymes. Liu et al. (2007) compared the transcriptional response in *Medicago truncatula* to different AM fungi. A core set of genes some of them associated with defense mechanisms, were induced in response to the different AM fungi, suggesting that gene induction specific to a single AM fungus might be rare. A diverse AM fungal assembly might be able to induce more defense-related genes rather than one fungus alone. Mycorrhizae also play an important role in selection of the microbial community by host and edaphic factors creates a specialized tree-mycorrhiza-rhizobacterial metaorganism that deploys the genome resources of all symbionts to explore soils according to (Cumming et al. 2015; Fig. 9.5).

9.4 Mycorrhizae in Crop Improvement

Beneficial micro-organisms, including soil-borne symbionts, N_2-fixing bacteria, and AM fungi, provide minerals to plants are directly implicated in crop production. Mycorrhizae provide mutualistic symbiosis between some soil-borne fungi and plant roots (Frank 1885). Symbiotic fungi are common worldwide in all soil types and climates; AM fungi (Morton and Benny 1990), have coexisted and coevolved with plants for about 400 million years (Malloch et al. 1980; Pirozynski and Dalpé 1989). This means most of the root systems of agricultural/horticultural

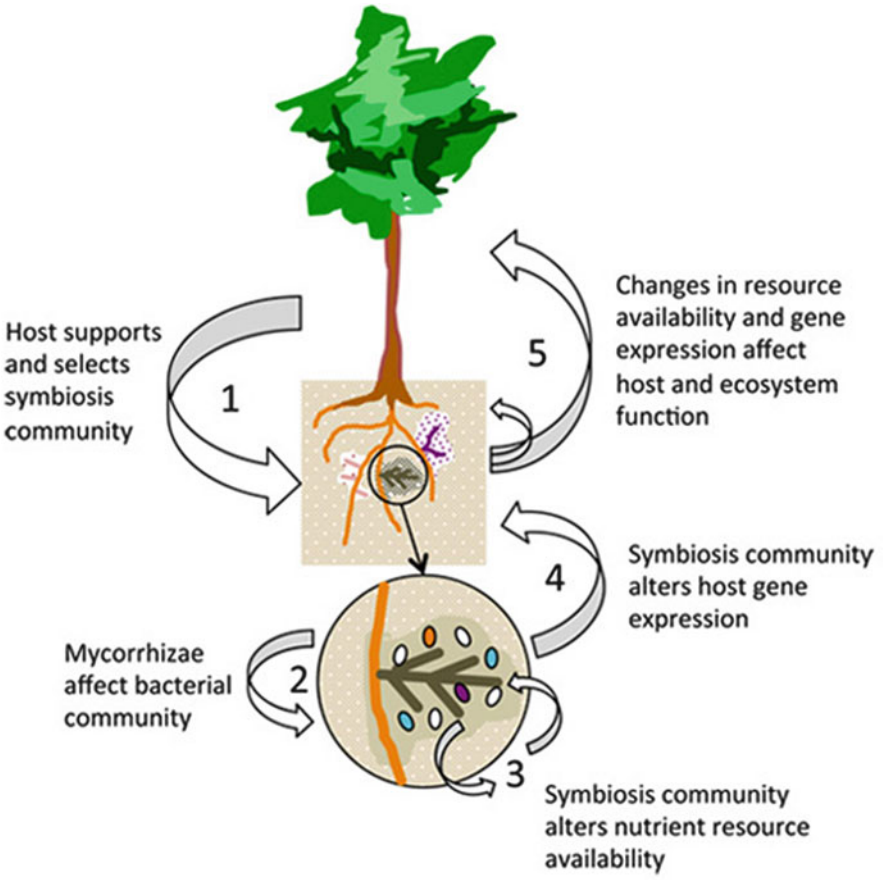


Fig. 9.5 Role of mycorrhizae in selection of the microbial community by host and edaphic factors creates a specialized tree-mycorrhiza-rhizobacterial metaorganism that deploys the genome resources of all symbionts to explore soils (Cumming et al. 2015)

plants and crops are colonized by AM fungi (Harley and Harley 1987; Sieverding 1991).

In order to take advantage of mycorrhizae through innovative cropping systems, the following three conditions must be met:

1. We need indicators of AM fungi population and AM development on plants. We need to be able to characterize AM fungi and mycorrhizae as easily as soil richness in terms of exchangeable cations. This may permit AM fungi characterization to be integrated with an agronomic diagnosis approach (Doré et al. 1997). This would help us to discern when low mycorrhizal development leads to low production.
2. We need a better knowledge of the relationship between cropping systems and AM fungi so that mycorhizae can be integrated into crop models. Innovations in

cropping systems will rely more and more on scenarios predicted by models (Meynard 1998; Meynard et al. 2001; Boiffin et al. 2001); experimentations "in silico" will allow us to predict the consequences of different combinations of cropping practices under various climates.

3. We need economic and social conditions to promote sustainable production instead of short-term solutions that meet immediate needs. Considerations for a better valorization of mycorrhizae, the separation between cropping and intensive animal production, sometimes out of ground in some parts of the world, shows its limits, and the use of pesticides as the preferential means of controlling the ecosystem, appear to constitute a no-win game of sorcerer's apprentice (Plencette et al. 2005).

Ectomycorrhizal inoculum is easily produced for application in forest nurseries, but the necessity of AM inoculum production via a host plant is still an obstacle to ample utilization of AM fungi in agricultural crops. Nevertheless, progress is being made in this area and some commercial inoculum is currently marketed in the USA. Some of the important practical applications of mycorrhizae are: (a) in soil/substrate (including transplanting media) that are constantly fumigated or receiving high rates of fungicides to eliminate/reduce soil borne pathogens, such as in horticultural crops and fruits; (b) in revegetation of eroded or mined areas (extreme pH; metal toxicity; low organic matter content, natural AM inoculum, and overall fertility); and (c) in arid and semi-arid regions. With increasing concerns about excessive nutrient application to the environment, the use of mycorrhizal symbioses to promote plant growth while reducing the inputs of fertilizer and pesticides may have great potential for citrus and vegetable crops, which respond very well to inoculation (Muchovej 2001).

9.5 Effect of Nanoparticles on Mycorrhizae and Pathogenic Fungi

Nanoparticles are tiny particles which measures from 1 to 100 nm in diameter, having grater surface area, yielding greater proportion of surface atoms and resulting in greater surface reactivity. Nanoparticles have unique physiochemical properties resulting in increased use in wide range of technical applications and consumer products. Silver nanoparticles are currently used in textiles, personal care products, food storage, home appliances, paints etc. Iron oxide nanoparticles are widely used in biomedical and environmental applications. Plants are essential component of terrestrial ecosystem and play crucial roles in the fate and transport of nanoparticles throughout the environment via uptake and bioaccumulation. Hence, plant responses to nanoparticles are of great interest and aid in understanding of the consequences of introducing into ecosystem. It has been reported that silver nanoparticles has potential to decrease plant biomass, reduce the length of shoots and roots of *Triticum aestivum*, failure in development of root hairs in

Lolium multiflorum. It exhibits highly vacuolated and collapsed cortical cells and broken epidermis and root cap. Arbuscular mycorrhizae are ubiquitous and exhibit symbiotic association with 90% land plants. it has been found that AM can alleviate metal stress on *Phragmites australis* and *Iris pseudocorus* via transformation of cationic copper into copper nanoparticles. Silver nanoparticles are toxic to plants but exhibit growth promoting effect on AM. Feng et al. (2013) found that, the elevated levels of silver nanoparticles has ability to enhance the effectiveness of AM for stress elevation in host plants. Glomalin is a glycoprotein produced abundantly on hyphae and spores of AM fungi. It has been reported that FeO nanoparticles exhibit potential to inhibit mycorrhizal plant growth by adversely affecting AM fungi excreted glomalin. Wang et al. (2016) reported that high level of contamination by zinc oxide nanoparticles causes toxicity to AM symbiotic association, but AM fungi help in allegation ZnO nanoparticle induced phytotoxicity by decreasing Zn bioavailability and accumulation, shoot partitioning by Zinc, ROS production, increasing mineral nutrition and increasing antioxidant capacity.

9.6 Conclusion

Mycorrhizae are the potential tools for environmentally sustainable approach to decrease pathogenic fungi. Mycorrhizae are play important roles in the growth and development of plants directly and indirectly through several mechanisms. The pathogenic fungi and the mycorrhizal fungi compete for the available nutrients and resources. Pathogenic fungi colonization decreased due to mycorrhizal fungi colonization. Out of all the mycorrhizae studied and discovered, AM is the most studied type and most of the researches were done on it. The studies have proven that infected plants when treated with AM fungi its colonization deceases the pathogenic fungi colonies. The discovery of many traits and genes that are involved in the beneficial effects of mycorrhizae may result in a better understanding of the performance of bioinoculants in the field and provides the opportunity to enhance the beneficial effects of mycorrhizal strains by genetic modifications.

References

Abbott LK, Robson AD (1981) Infectivity and effectiveness of five endomycorrhizal fungi: competition with indigenous fungi in field soils. Aust J Agric Res 32:621–630

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 1:1–10

Bagyaraj DJ (2014) Mycorrhizal fungi. Proc Indian Nat Sci Acad 80:415–428

Boiffin J, Malézieux E, Picard D (2001) Cropping systems for the future. In: Nosberger J, Geiger HH, Struik PC (eds) Crop science: progress and prospects. CAB International, Oxford, pp 261–279

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant- fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046
Budi SW, Van Tuinen D, Martinotti G, Gianinazzi S (1999) Isolation from the *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soilborne fungal pathogens. Appl Environ Microbiol 65:PMC91693
Cardon ZG, Whitbeck JL (2007) The rhizosphere. Elsevier Academic Press, Burlington, MA, p 235
Cumming JR, Zawaski C, Desai S, Collart FR (2015) Phosphorus disequilibrium in the tripartite plant-ectomycorrhiza-plant growth promoting rhizobacterial association. J Soil Sci Plant Nutr 15(2):464–485. https://doi.org/10.4067/S0718-95162015005000040
Davis RM, Menge JA (1981) *Phytophthora parasitica* inoculation and intensity of vesicular-arbuscular mycorrhizae in citrus. New Phytol 87:705–715
Doré T, Sebillotte M, Meynard JM (1997) A diagnostic method for assessing regional variations in crop yield. Agric Syst 54:169–188
Dressier RL (1993) Phylogeny and classification of the orchid family. Cambridge University Press, Cambridge
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 47:107–118
Frank AB (1885) Uber die auf wurzelsymbiose beruhende ernärhung gewisser bäume durch unterirdische pilze. Ber Deutsch Bot Gesell 3:128–145
Freudenstein JV, Doyle JJ (1994) Plastid DNA, morphological variation, and the phylogenetic species concept: the *Corallorhiza maculata* (Orchidaceae) complex. Syst Bot 19:273–290
Geerinck D (1992) Flored'Afrique Centrale Orchidaceae 2. Jardin Botanique rationale de Beige
Genre A, Ortu G, Bertoldo C, Martino E, Bonfante P (2009) Biotic and abiotic stimulation of root epidermal cells reveals common and specific responses to arbuscular mycorrhizal fungi. Plant Physiol 149:1424–1434
Giri B, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmuller R, Varma A (2005) Mycorrhizosphere: Strategies and Functions. In: Buscot F, Varma A (eds) Microorganisms in Soils: Roles in Genesis and Functions. Springer-Verlag Berlin, Heidelberg 213–252
Gutjahr C, Casieri L, Paszkowski U (2009) *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. New Phytol 182:829–837
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, Toronto
Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153:335–344
He XH, Critchley C, Bledsoe CS (2003) Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). Crit Rev Plant Sci 22:531–567
Jaiti F, Meddich A, Hadrami I (2007) Effectiveness of arbuscular mycorrhizal fungi in the protection of date palm (*Phoenix dactylifera* L.) against bayoud disease. Physiol Mol Plant Pathol 71:166–173
Jansa J, Smith FA, Sally ES (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytol 177:779–789
Lin X, George E, Marschner H (1991) Extension of the phosphorus depletion zone in VA mycorrhizal white clover in a calcareous soil. Plant Soil 136:41–48
Lioussanne L, Jolicoeur 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
Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529–544

Malloch DW, Pirozynski KA, Raven PH (1980) Ecological and evolutionary significance of mycorrhizal symbiosis in vascular plants (a review). Proc Natl Acad Sci USA 77:2113–2118
Matsubara Y, Tamura H, Harada T (1995) Growth enhancement and Verticillium wilt control by vesicular-arbuscular mycorrhizal fungus inoculation in eggplant. J Jpn Soc Hortic Sci 64:555–561
Meynard JM (1998) La modélisation du fonctionnement de l'agrosystème, base de la mise au point d'itinéraires techniques et de systèmes de culture. In: Biarnès A, Fillonneau C, et Milleville P (eds) La gestion des systèmes de culture: regards d'agronomes. ORSTOM, pp 29–54
Meynard JM, Doré T, Habib R (2001) L'évaluation et la conception de systèmes de cultures pour une agriculture durable. CR Acad Agric Fr 87:223–236
Mohammadi K (2011) Soil, plant and microbe interactions. Lambert Academic Publishing, Berlin, p 113
Mohammadi K, Khalesro S, Sohrabi Y, Heidari G (2011) Beneficial effects of the mycorrhizal fungi for plant growth. J Appl Environ Biol Sci 1:310–319
Moore D, Robson GD, Trinci APJ (2011) 21st century guidebook to fungi. Cambridge University Press, Cambridge. isbn:9780521186957
Morton JB, Benny JD (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 emendation of Glomaceae. Mycotaxon 37:471–491
Muchovej RM (2001) Importance of mycorrhizae for agricultural crops. 1 SS-AGR-170
Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol Evol 10:407–411
Norman JR, Atkinson D, Hooker JE (1996) Arbuscular mycorrhizal fungal induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. Plant Soil 185:191–198
Olah B, Briere C, Becard G, Denarie J, Gough C (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J 44:195–207
Oldroyd GED, Harrison MJ, Paszkowski U (2009) Reprogramming plant cells for endosymbiosis. Science 324:753–754
Parry DW, Jenkinson P, McLeod L (1995) Fusarium head blight (scab) in small grain cereals- a review. Plant Pathol 44:207–238
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 USA 99:13324–13329
Perotto S, Angelini P, Bianciotto V, Bonfante P, Girlanda M, Kull T, Mello A, Pecoraro L, Perini C, Persiani AM, Saitta A, Sarrocco S, Vannacci G, Venanzoni R, Venturella G, Selosse MA (2013) Interactions of fungi with other organisms. Plant Biosys. https://doi.org/10.1080/11263504.2012.753136
Pirozynski KA, Dalpe Y (1989) Geological history of the Glomaceae with particular reference to mycorrhizal symbiosis. Symbiosis 7:1–36
Plencette C, Clermont-Dauphin C, Meynard JM, Fortin JA (2005) Managing arbuscular mycorrhizal fungi in cropping systems. Can J Plant Sci 85:31–40
Powell JR, Parrent JL, Hart MM, Klironomos JN, Rillig MC, Maherali H (2009) Phylogenetic trait conservatism and the evolution of functional tradeoffs in arbuscular mycorrhizal fungi. Proc R Soc B 276:4237–4245
Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398
Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defense responses to *Phytophthora infection* in tomato plants. J Exp Bot 53:525–534

Price N, Roncadori R, Hussey R (1989) Turgor, solute import and growth in maize roots treated with galactose. New Phytol 31:1095–1103

Selosse M-A, Le Tacon F (1998) The land flora: a phototroph-fungus partnership? Trends Ecol Evol 13:15–20

Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Technical cooperation, Eschborn, p 371

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

Smith S, Read D (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, San Diego

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1995) Altered growth of *Fusarium oxysporum* f sp. chrysanthemi in an in-vitro dual culture system with the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5:431–438

Trotta A, Varese GC, Gnavi E, Fusconi A, Sampo S, Berta G (1996) Interactions between the soilborne root pathogen *Phytophthora nicotianae* var. parasitica and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209

Van Wees SCM, van der Ent S, Pieterse CMJ (2008) Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol 11:443–448

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 e A soil microcosm experiment. Chemosphere 147:88–97

White JM (1984) Competition for infection between vesicular-arbuscular mycorrhizal fungi. New Phytol 97:427–435

Windels C (2000) Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathol 90:17–21

Yang S, Pfister D (2006) Monotropa uniflora plants of eastern Massa-chusetts form mycorrhizae with a diversity of russulacean fungi. Mycologia 98:535–540

Yano K, Yamauchi A, Kono Y (1996) Localized alteration in lateral root development in roots colonized by an arbuscular mycorrhizal fungus. Mycorrhiza 6:409–415

Chapter 10
Arbuscular Mycorrhizal Fungi as Biocontrol Agents for Parasitic Nematodes in Plants

Khursheed Ahmad Wani, Javid Manzoor, Razia Shuab, and Rafiq Lone

Abstract The use of synthetic fertilizers and pesticides has not only caused damage to environment but has caused detrimental impacts on the health of people. In order to feed the ever growing population and prevent environmental contamination and decrease the impact on human health organic farming is being promoted all over the world. The use of Arbuscular mycorrhiza fungi to boost agricultural productivity is considered a better alternative as it has strong influence on plant interactions by aiding plants in resource acquisition, disease suppression, and tolerance to soil pollution and play a decisive role in plant development. It also enhance the supply of water and nutrients (phosphate and nitrogen), to the host plant. In return, up to 20% of plant-fixed carbon is transferred to the fungus; hence the nutritional exchange is bidirectional. AMF acts as a biocontrol agent for various crops and thus reduces the burden of pesticides in agro-ecosystems. Advance research is needed to develop farming systems that optimize the use of natural resources such as mycorrhizal fungi for sustainable agricultural production. The present chapter is an attempt to study the role of AMF in controlling different plant parasitic nematodes along with its important advantages for the crop production.

10.1 Introduction

The increase in the yield of agriculture has been achieved by the introduction of inorganic fertilizers and pesticides (Reddy and Wang 2011). Today, agricultural practices are dependent on the chemical fertilizers and pesticides and use of

K.A. Wani
Department of Environmental Science, ITM University, Gwalior, India

J. Manzoor
Department of Environmental Science, Jiwaji University, Gwalior, India

R. Shuab
Department of Botany, University of Kashmir, Srinagar, Jammu and Kashmir, India

R. Lone (✉)
Department of Botany, SBBS University, Khiala, Jalandhar, Punjab 144030, India
e-mail: rafiqlone@gmail.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_10

nitrogen, phosphorus and potassium based fertilizers increased the crop productivity to large extent. However, increased dosage of chemical fertilizers caused serious effects on soil fertility that caused not only environmental pollution but also killed the beneficial microorganisms present in soil and thus increased resistance in pests against these pesticides. The poisonous chemicals which are applied in agricultural orchards are absorbed by plants; enter the food chain through vegetables and cereals causing many health problems. To overcome the problems a sustainable agricultural approach by involving microbial technology such as Mycorrhizae as biofertilizer and biocontrol agents is required (Dwivedi and Padmanabh 2002) as they play a fundamental role in nutrients fixing, solubilizing and mobilizing nutrients. Mycorrhizae have significant effect on the rhizosphere as they interact with other soil biota such as phosphate solubilizing bacteria, plant growth promoting rhizobacteria, plant pathogens and other bioagents, which results in a significant positive or negative effects on plant growth (Paulitz and Linderman 1991) hence, play a important role in sustainable farming system because AMF are efficient when nutrient availability is low and nutrients are bound to organic matter and soil particles. Arbuscular mycorrhizal fungi are obligate root symbionts that colonize more than 80% of all land plant species. They increase plant growth through improved nutrient uptake in exchange for photosynthetic carbon from their host (Smith et al. 2010). Hence, AMF has a strong potential to increase the agricultural productivity and may help in conservation of environment by reducing the burden of fertilizers and pesticides.

AMF provide their favourable services to almost all terrestrial ecosystems, from grasslands to forests, deserts and agro-ecosystems as it is present in one or the other species in these ecosystems. The formation of symbiotic relationships with the majority of land plants, including many important crops is the key for its establishment in such ecosystems (Smith and Read 2008) and generally provide nutrients, especially phosphorus, to plants in exchange for plant carbohydrates (Smith and Read 2008). Further, they can provide protection against pathogens (Sikes et al. 2009) and drought (Auge 2001). With the advent of modern genetic engineering, AMF is tested for many other crops and success rate is quite appreciable, thus novel transgenic crop plants with superior yields and improved traits for resistance to insect pests and pathogens, tolerance to herbicides, and improved ability to endure environmental stress has been created (Sharma and Srivastava 2008). The improvement in soil structure and soil properties by mixing AMF enhances the sustainability of waste lands (Wilson et al. 2009), and by reducing nutrient losses after rain-induced leaching events that will help in the restoration of wastelands and reclamation of barren areas (Van der Heijden 2010) round the year as different species are active during different seasons. Merryweather and Fitter (1998), that makes it suitable for different plant species under different environmental conditions (Ravnskov and Jakobsen 1995). Several studies indicated that diverse AMF communities may improve plant productivity and ecosystem functioning as observed in (van der Heijden et al. 1998; Vogelsang et al. 2006). AMF may become significant in escalating crop productivity in acid soils because they may stimulate Phosphorus uptake in highly P-fixing conditions. Enhanced uptake of P is generally regarded as the most significant benefit that AMF provide to their host plant, and plant P status

is often the main controlling factor in the plant-fungal relationship (Graham 2000). The present necessity in agriculture for high yields as quickly as possible may be an ongoing necessity for the future of food production. Smith and Read (1997a, b) pointed out that incorporation of cultural practices will increase AMF diversity. Sustainable production of food crops in the tropics is often severely constrained by the fragility of soils, being prone to several forms of degradation. Making better use of the biological resources in these soils can contribute to enhanced sustainability. Mycorrhizal fungi constitute an important biological resource in this respect. Therefore, the aim of the present chapter is to study the role of AMF in controlling different plant parasitic nematodes along with its important advantages for the crop production.

10.2 AMF and Organic Agriculture

The association of AMF brings significant changes in the host plant and its environment by changing the rhizosphere level, soil structure, carbon deposition, microbial diversity and in part through changes in exudation of roots. The out-come of plant interactions with other organisms, including pathogens and beneficial microbes is due to then shifts in the microbial communities of the rhizosphere (Berta et al. 2002, 2005; Artursson et al. 2006; Lendzemo et al. 2007; Cipollini et al. 2012; Effmert et al. 2012) with multiple modifications in the rhizosphere within the host plant. Therefore, it is believed that introduction of AMF for organic agriculture has diverse benefits. Organic farming by AMF will preserve and conserve the environment's balance, increases natural resources and develops healthier and better products. Agricultural systems use large quantities of inorganic fertilizers and pesticides is a method to alleviate food shortages. However, the introduction of heavy chemicals in agricultural systems cause serious problems, such as disturbance of agricultural ecosystems, and have high environmental costs. The degenerative effects of intensive farming practices on soil fertility and ecological balance are surfacing which needs immediate attention for sustaining the productivity rate (Narayanan 2005; Dubey and Shukla 2014). However, the major problem in most developing countries is the poor nutritional status of soils due to less content of the organic matter (Narayanan 2005; Dubey and Shukla 2014). AMF is seen as an ecological production management system that will promote and enhance biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony. The aim of introduction of AMF into agricultural ecosystems is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people (National Organic Standards Board 1997). However, a lot will depend on the efficiency of AMF inputs in the promotion of the productivity on the organic contents of the soil. Mycorrhizal fungi are now common in agricultural systems and are particularly relevant for organic agriculture because they can act as natural fertilisers, enhancing plant yield. Moreover, mycorrhizal

fungi can directly and indirectly contribute to plant productivity in organic farming systems (Van der Heijden et al. 2008). Low-input organic farming systems have increasingly aroused interest due to their focus on natural resource conservation and reduction of environmental degradation. The characteristics of general principals of organic farming include: (1) elimination of synthetic biocides; (2) addition of organic fertilizers to the soil, including farmyard manure, compost and crop residue, and slow release mineral fertilizers such as rock phosphate; and (3) crop rotation practices must be encouraged (Van der Heijden et al. 2008). Organic farming relies heavily on active soil microbial communities and AM fungi play a vital role in agro ecosystem function. It has been reported that compared to conventional systems of organic farming, AMF has increases microbial colonization, propagule numbers, and species diversity (Eason et al. 1999; Oehl et al. 2004; Ryan et al. 2000).

10.3 Role of AMF in Soil Improvement

The health of plants is closely linked to soil health, managing the soil in different ways that conserve and enhance the soil biota can improve crop yields and quality. Agricultural management also has profound effects on soil communities (Zadehbagheri et al. 2014b). A varied soil community will not only help prevent losses due to soil-borne pests and diseases but also speed up decomposition of organic matter and toxic compounds, and improve nutrient cycling and soil structure. Microorganisms are the most abundant members of the soil biota. They include species responsible for nutrient mineralization and cycling, biological control agents against plant pests and diseases, species that generate substances capable of modifying plant growth, and species that form mutually beneficial symbiotic relationships with plant roots (SP-IPM 2004). The most abundant members of the vast community of soil organisms that develops mutually beneficial relationships with plant roots and contributes majorly to plant growth are called mycorrhizal fungi. They are versatile organisms with complex ecological ramifications in the soil system that has been complicated to study and understand (Kapoor et al. 2004; Cavagnaro et al. 2006). The phytocentric concept of AMF that has prevailed since the naming of these organisms is being replaced by a holistic vision recognizing that AMF are a key element of soil functioning and health rather than a plant root component (Zadehbagheri et al. 2014a, b). The most common and best known of these associations are the AMF (Powell and Bagyaraj 1984). AMF have been suggested to improve biodegradation of persistent organic pollutants because of the immense size and very high surface interface with soil. AM fungi are of importance as they play a vital role in metal tolerance (Del Val et al. 1999) and accumulation (Zhu et al. 2001; Jamal et al. 2002). AMF associations have a direct effect on soil structure, which is especially important in mixed culture systems, where cultivations and low levels of soil organic matter tend to result in damaged soil structure. AMF association with crop components in mixed

culture systems have been reported to have a great impact in soil structure (Borie et al. 2000; Franzluebbers et al. 2000; Wright and Anderson 2000; Rillig et al. 2003; Rillig 2004). The essential ecological role played by AMF is their capacity to influence directly the diversity and composition of the aboveground plant community confirmed that plant species richness can be altered not only by climatic and edaphic factors, but also by soil microbial assemblages (Van der Heijden et al. 1998; Scheublin et al. 2007; Facelli et al. 2010). In addition, they improve plant growth, help in contaminant removal, reduce the need for fertilizer application in commercial plant production, and improve the soil structure and health. Although, relatively few specific plant-fungus combinations have been studied for their efficacy and application in remediation and resource conservation, the existing data on the benefits for mycorrhiza are promising.

10.4 Mechanism of Inhibition by AMF

Different mechanisms are reported to explain bio-control by AMF including biochemical changes in plant tissues, microbial changes in rhizosphere, nutrient status, anatomical changes to cells, changes to root system morphology and stress alleviation (Hooker et al. 1994). Various workers have demonstrated the protective effect of mycorrhizal symbioses against root pathogenic fungi (Caron 1989; Dehne 1982). The complex interactions between pathogens, AMF and plant are consequences of disease reduction within host plants colonized by AMF (Harrier and Watson 2004). The damage caused by soilborne pathogens is inhabited by AMF symbiosis (Azcon-Aguilar et al. 2002). *Phytophthora parasitica* propagation decreases when tomato root is colonized by *Glomus mosseae* and *P. parasitica* compared with non-mycorrhizal tomato roots (Cordier et al. 1996). The contribution of phosphate by AMF lessen the damage caused by *P. parasitica* in tomato (Trotta et al. 1996). The time required by *Ganoderma boninense* to infect and kill oil palm plant and the seedlings is greatly reduced (Rini 2001). AMF has shown no indirect interaction with soilborne pathogen through antagonism, mycoparasitism and or antibiosis (Harrier and Watson 2004). AMF may enhance host tolerance to pathogen by increasing the uptake of essential nutrients rather than phosphorus which are otherwise deficient in the non-mycorrizal plants (Gosling et al. 2006). The nutrient uptake resulted in more dynamic plants and make the plant itself may be more resistant or tolerant to pathogen attack (Linderman 1994). The uptake and translocation of nitrogen by hyphal fungus is controlled by host plant's demand for N (Hawkins and George 2001). AMF increase host resistance of pathogen attack to compensate the loss of root functional activities and biomass caused by soil borne pathogens (Linderman 1994) including fungi and nematodes (Cordier et al. 1996). AM fungi enhanced uptake of Zn, S and Ca (Clark and Zeto 2000) and also Iron (Fe) acquisition has been enhanced, apart from phosphorus and Nitrogen uptake. It is found that AM plants that are grown at low pH had higher Fe gain than AM plants grown at high pH (Treeby 1992). Manganese gain generally was lower in AM

plants compared to non AM plants (Azaizeh et al. 1995). Copper and zinc concentrations increased in leaves of AM soybean plants, sulfur acquisition was enhanced in sorghum colonized by *Glomus fasciculatum* (Raju et al. 1990), boron content was enhanced in AM maize shoot and calcium (Ca), sodium (Na) and magnesium (Mg) was also increased compared to the non AM *Gigaspora gigantia* soybean plants in low Phosphorus.

Plants colonized by AMF vary from non-mycorrhizal plant in rhizosphere microbial community that cause changes in root respiration rate quality and quantity of the exudates (Marschner et al. 2001) is another proposed mechanism. Few researchers believe that that AMF changes the composition of functional groups of microbes in the mycorrhizosphere, including the numbers and/or activity of pathogens antagonists (Secilia and Bagyaraj 1987). Fluctuations in the functional groups of microbes, including more facultative anaerobic bacteria in mycorrhizosphere of AMF colonized *T. Subterraneum* has been observed. The total number of bacteria isolated from rhizoplane of *T. subterraneum* and *Zea mays* increased due to AMF colonization (Meyer and Linderman 1986). Physical competition between endomycorrhizal fungi and rhizosphere microorganisms to occupy more space in the root architecture is one of the mechanism proposed to explain the interaction between AMF and soil microorganisms (Bansal and Mukerji 1996). Morphological and anatomical changes as observed by Dugassa et al. (1996) in tomato and cucumber can be one more explanation to inhibit pathogens. A limited data in support for the carbon compounds received by the root has been cited in literature (Smith and Read 1997a, b). The higher carbon demand may inhibit the pathogen growth when AMF have primary access to the photosynthate (Linderman 1994).

10.5 Arbuscular Mycorrhizae as Biocontrol Agents of Plant Parasitic Nematodes

Plant-parasitic nematodes, including endoparasitic nematodes and AMF often survive collectively in the rhizosphere colonizing the same area in roots of host plants. The association between AMF and nematodes (Hussey and Roncadori 1982; Elsen et al. 2003; de la Peña et al. 2006) and nematode reduction (Sankaranarayanan and Sundarababu 1994; John and Bai 2004; Kantharaju et al. 2005; Siddiqui and Akhtar 2007), no effect (Hasan and Jain 1987) or an increase in numbers of nematodes (Atilano et al. 1981) has been reported from earlier times. Various studies confirmed that AMF enhances host tolerance or resistance in many plant/nematode systems and induce systemic resistance against plant-parasitic nematodes in the roots (Elsen et al. 2008). Marro et al. (2014) reported that plant-parasitic nematode *Nacobbus aberrans* induces gall formation in the roots and causes severe losses to diverse crops. Few nematodes of this group show inclination for certain hosts with different behaviour that make nematode management

difficult. The authors argued that a possible biological control alternative to decrease the damage caused by this species may be the use of arbuscular mycorrhizal fungi (AMF). The consequence of *Glomus intraradices* on tomato plants inoculated with the nematode at transplanting. The use of AMF favoured tomato biomass and decreased the number of galls on the plants inoculated with the nematode at transplanting.

AMF is used as biocontrol agents to decrease infestation by root-knot nematodes as it is believed that AMF and plant parasitic nematodes compete with each other for the same site. The population of *Tylenchulus semipenetrans* infesting citrus is controlled by the dose of AM fungus of 500 chlamydospores per tree (Pandey et al. 2004). Rodriguez Romero and Jaizme-Vega (2005) reported that micro-propagated plants of banana (*Musa acuminate*) cv. Grand Naine were inoculated with *Glomus manibotis* initially and then by *Meloidogyne javanica* that reduced the number of galls and the population of *M. javanica* with no harmful effect on root colonization by the mycorrhizal fungus. The interactive effect of mycorrhizal fungus, *Glomus fasciculatum* with root knot nematode, *Meloidogyne incognita* and their effect on tomato was studied by Shreenivasa et al. (2007). Nematode population, number of galls and root knot index was significantly reduced by *G. fasciculatum* and increased the growth, phosphorous uptake, plant biomass and productivity of tomato compared to plants inoculated with nematode alone. Hajra et al. (2013) studied the biocontrol of nematodes by arbuscular mycorrhizal (AM) fungi and reported significant variations in different parameters. The mycorrhized plants showed increase in leaf area and plant height than non-mycorrhizal plants, but mycorrhizal plants exhibited a sharp drop off in nematode- infected plants due to xylem vessels damage. The capability of mycorrhizal fungi, oil palm bunch refuse and sawdust mulches on banana growth and nematode infection has been investigated by Omolara (2014). Sawdust mulch enhanced leaf area by 215%, mycorrhizal fungi by 234% and oil palm bunch refuse by 267%.

Nematodes are diverse group comprising free-living nematodes and plant and animal parasites that are present all over the world in various habitats (Ferraz and Brown 2002). Many species of plant-parasitic nematodes (PPN) can act as pests on a wide range of economically important agricultural crops. The use of biological control organisms, such as AMF is an environmentally friendly alternative to manage PPN (Bajaj et al. 2015, 2017). AMF can may alleviate plant stress caused by abiotic and biotic factors, including PPN (Gianinazzi et al. 2010; Singh et al. 2011; Vos et al. 2012a). The biocontrol effect of AMF was also seen in different plant species and against many pathogens, although, successful biocontrol has also been observed against aboveground pathogens such as *Alternaria solani* in tomato (Harrier and Watson 2004; Whipps 2004; Fritz et al. 2006; Pozo and Azcón-Aguilar 2007; Jung et al. 2012). Necrotrophic and biotrophic pathogens were reported to be inhibited by AMF, either directly or indirectly (Veresoglou and Rillig 2012). Greenhouse and field experiments indicated defensive effects against PPN by AMF in plants such as banana, coffee and tomato (Calvet et al. 2001; Vos et al. 2012b; Alban et al. 2013; Koffi et al. 2013).

AMF significantly decreased seed germination of a few obligate root parasitic plant species like *Strigam hermonthica* (Lendzemo et al. 2005, 2006; Hearne 2009; Gworgwor and Weber 2003) and *Orobanche Cumana* (Louarn et al. 2012) and thus have a potential in biocontrol against obligate root parasitic weeds. Li et al. (2012) reported, inoculation with AM fungi inhibited haustorium formation in facultative root hemiparasitic *Pedicularis tricolor*. Further, phosphorus transfer from the host plant into *P. tricolor* and *Pedicularis rex* was significantly decreased. The growth of hemiparasites was inhibited after AM inoculation, suggesting that AMF have a potential in the management of *Pedicularis* species (Li et al. 2013).

The effects of *Fusarium oxysporum*, a soil-borne biocontrol agent (BCA) was studied against *Striga hermonthica*, on fungal and arbuscular mycorrhizal fungal (AMF) taxa in rhizospheic clay and sandy soil of maize. 'Foxy-2' that was used against *S. hermonthica* promoted total fungal abundance and stimulated AMF *Gigaspora margarita* abundance. No adverse effects were shown by 'Foxy-2' on indigenous rhizosphere fungal communities indicating its environmental safety as BCA against *S. hermonthica* (Zimmermann et al. 2016).

Present study deals with the biocontrol of *Fusarium* wilt of chickpea using arbuscular mycorrhizal fungi (AMF) *Glomus hoi* (Gh), *Glomus fasciculatum* (Gf) and *Rhizobium leguminosorum Biovar*. (Rl), which are the important members of rhizosphere and biological control agents, like AMF, Gh, Gf and RI were studied on both the patho- system of *Fusarium oxysporum* f. sp. ciceris (Foc) and chickpea (*Cicer arietinum*). The differences were exhibited on colonization and nodulation of two biocontrol agent reciprocal interactions. The decrease in nodulation of Rl particularly and colonization of AMF significantly decreased in treatment of Foc +AMF than control. The single biological control agent inoculations were more effective than dual inoculations (AMF+Rl) and the morphological parameters of chickpea showed decrease in treatments which present Foc. In addition to this, all biological control agent increased total contents of P and N in treated plants compared to controls (Singh et al. 2014).

The obligate symbionts that colonize the roots of the most cultivated plant species are known as AMF and almost all types of ecological situations may be found naturally in most of the species. Endoparasitic nematodes and arbuscular mycorrhizal fungi, together colonize the same area of the rhizosphere roots of host plants during interaction. The members of the microbial populations like AM fungi and root knot of the plant rhizosphere compete with each other for the same site in rhizosphere. Hence, AM fungi can be used as biocontrol agents to reduce infestation by root-knot nematodes. Systemic resistance against plant-parasitic nematodes in the roots of higher plants improved host nutrition.

Antagonism has been demonstrated and believes to be the result of or may be due to improved host nutrition (Youssef and El-Nagdi 2015). *Verticillium dahliae* Kleb is a vascular pathogen that alters water status and growth of pepper plants and causes drastic reductions in yield. Its control is difficult because it can survive in field soil for several years. The application of AMF as bioprotector agents against *V. dahliae* is an alternative to the use of chemicals which, in addition, is more respectful with the environment. Some AMF can improve the resistance of

Capsicum annuum L. against *V. dahliae*. This is especially relevant for pepper cultivars (i.e. cv. Piquillo) that exhibit high susceptibility to this pathogen. Compared with non-mycorrhizal plants, mycorrhizal pepper can exhibit more balanced. A balanced antioxidant metabolism in leaves of mycorrhizal pepper has been observed after pathogen inoculation that delays the development of disease symptoms and photosynthesis decrease in *Verticillium*-inoculated plants. The higher deposition of lignin in xylem vessels of stem cells was shown as compared to non-mycorrhizal plants. AMF has been used as bioprotector agent against *V. Dahlia* and improved the resistance of *Capsicum annuum L* against *V. Dahlia*. Non-mycorrhizal plants of the arbuscular and ectomycorrhizae out of the known mycorrhiza are the most abundant and wide spread that promote plant growth by enhancing nutrient acquisition and promoting growth hormones. The increase in the resistance in plants against plant pathogens and surface area of root system for better absorption of nutrient from soil may be achieved by using AMF, hence may be used as biofertilizer and as biocontrol agents (Goicoechea et al. 2010).

Sui et al. (2014) observed AM colonization in roots of *P. kansuensis*, although much lower than those of its adjacent host species of hemiparasite. The relation between AM levels and the number of haustoria was negative for field samples of the hemiparasite. A significant reduction in plant dry weight (DW) with strong suppression in the production of haustorium and marked reduction in the survival rate of *P. kansuensis* after inoculation with AM fungi was observed. On the other hand, inoculation with *G. mosseae* enhanced root DW and whole plant DW of parasitized host plants. A positive repressive effect of AMF on growth performance of *P. kansuensis*. Leta and Selvaraj (2013) pointed out that *G. aggregatum* and *T. harzianum* ATh1 isolate can block the severity of disease caused by *S. cepivorum* in onion. Use of these bio-control agents could be promoted as an active component of bio-intensive Integrated Disease Management Program (IDMP), under organic mode. Vigo et al. (2000) confirmed that effects on numbers of infection loci on tomato root necrosis are one mechanism *via* which AMF achieve biocontrol of this pathogen. The rate of spread of necrosis within roots showed no changes caused by the AMF. Inoculation with the pathogen after 26 days at harvest revealed 61% of roots of noncolonized plants were necrotic compared with only 31% in AMF-colonized plants. Dehariya et al. (2015) investigated the effect of individual and co-inoculation of Trichoderma species and arbuscular mycorrhizal fungi on growth, mycorrhization, population of *Trichoderma*, and wilt disease severity in pigeon pea (*Cajanus cajan* L Mill sp.). Co-inoculation of Th and Myc gave highest growth and reduced severity of wilt disease of pigeon pea significantly. Myc alone was sufficient to promote growth and was effective in terms of disease suppression before pathogen inoculation. The shoot length, dry weight, phosphorus (P) uptake of plants, AMF colonization, spore density, and population of *Trichoderma*. Significant physiological changes take place in the host plant upon root colonization by AMF affecting the interactions with a wide range of organisms. Protective effects of the symbiosis against pathogens, pests, and parasitic plants have been described for different plant species, including agriculturally important crop varieties (Jung et al. 2012). A possible biological control alternative to reduce the damage caused

by *N. aberrans* may be the use of arbuscular mycorrhizal fungi. The effect of *Glomus intraradices* on tomato plants inoculated with the nematode at transplanting and three weeks later was tested. AMF colonization was higher in the presence of the nematode. The use of AMF favoured tomato biomass and reduce the number of galls and RF on the plants inoculated with the nematode at transplanting (Marro et al. 2014).

10.6 Conclusions

The minimal and no use of synthetic fertilizers in agriculture and the use of organic manure, to replenish the soil of its lost nutrients is known as organic farming. The research oriented towards use of AMF for control of pathogens or plant defence induction may replace pesticides and fertilizers in near future. The improvement in crop productivity and disease resistance is an indication for the same. The control of plant nematodes with the help of AMF will prevent losses arising due to various nematode pathogens. Changes in architecture, ability to increase the uptake of phosphorous by plant, metabolic profile fluctuations and defence compound accumulation occurrence makes AMF exceptionally different from other organic manures. In sustainable and organic agricultural systems, the role of AMF in maintaining soil fertility and bio-control of plant pathogens may be more important than in conventional agriculture where their significance has been marginalized by high inputs of agrochemicals. Therefore, the management and planned applications of AMF to improve growth of beneficial and important crops particularly in developing world with an understanding of exploiting AMF payback towards sustainable agricultural development is very important. It is believed that farming with AMF will be helpful in developing eco-friendly and cost effective plant disease management practices, will open and establish new avenues in the field of agriculture and industry.

References

Alban R, Guerrero R, Toro M (2013) Interactions between a root knot nematode (*Meloidogyne exigua*) and arbuscular mycorrhizae in coffee plant development (*Coffea arabica*). Am J Plant Sci 4:19–23

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

Atilano RA, Menge JA, Van Gundy SD (1981) Interactions between *Meloidogyne arenaria* and *Glomus fasciculatum* in grape. J Nematol 13:52–57

Auge RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azaizeh HA, Marschner H, Romheld V, Wittenmayer L (1995) Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. Mycorrhiza 5:321–327

Azcon-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Chuepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. From genes to bioproducts. Birkhauser, Berlin, pp 187–197

Bajaj R, Hu W, Huang Y, Chen S, Prasad R, Varma A, Bushley K (2015) The beneficial root endophyte *Piriformospora indica* reduces egg density of the soybean cyst nematode. Bioll Control 90:193–199

Bajaj R, Prasad R, Varma A, Bushley KE (2017) The role of arbuscular mycorrhizal fungi and the mycorrhizal-like fungus *Piriformospora indica* in biocontrol of plant parasitic nematodes. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza. Springer International Publishing AG, Cham, pp 43–56

Bansal M, Mukerji KG (1996) Root exudates and its rhizosphere biology. In: Mukerji KG, Singh VP, Dwivedi S (eds) Concepts in applied microbiology and biotechnology. Adita Books, New Delhi, pp 79–119

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Berta G, Fusconi A, Hooker JE (2002) Arbuscular mycorrhizal modifications to plant root systems: scale, mechanisms and consequences. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhaeuser, Basel, pp 71–85

Borie FR, Rubio R, Morales A, Castillo C (2000) Relationships between arbuscular mycorrhizal hyphal density and glomalin production with physical and chemical characteristics of soils under no-tillage. Rev Chil Hist Nat 73:749–756

Calvet C, Pinochet J, Hernández-Dorrego A, Estaún V, Camprubí A (2001) Field microplot performance of the peach-almond hybrid GF-677 after inoculation with arbuscular mycorrhizal fungi in a replant soil infested with root-knot nematodes. Mycorrhiza 10:295–300

Caron M (1989) Potential use of mycorrhizae in control of soil-borne diseases. Can J Plant Pathol 11:177–179

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability and soil aggregates in organic tomato production. Plant Soil 282:209–225

Cipollini D, Rigsby CM, Barto EK (2012) Microbes as targets and mediators of allelopathy in plants. J Chem Ecol 38:714–727

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902

Cordier C, Gianinazzi S, Gianinazzi-Pearson V (1996) Colonisation patterns of root tissues by *Phytophthora nicotianae* var. parasitica related to reduced disease in mycorrhizal tomato. Plant Soil 185:223–232

de la Peña E, Rodriguez-Echevarria S, van der Putten WH, 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

Dehariya K, Shukla A, Sheikh IA, Vyas D (2015) Trichoderma and arbuscular mycorrhizal fungi based biocontrol of *Fusarium udum* Butler and their growth promotion effects on pigeon pea. J Agric Sci Tech 17:505–517

Dehne HW (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115–1119

Del Val C, Barea JM, Azcòn-Aguilar C (1999) Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. Appl Soil Ecol 11:261–269

Dubey RK, Shukla N (2014) Organic farming: an eco-friendly technology and its importance and opportunities in the sustainable development. Int J Innov Res Sci Eng Tech 3:10726–10734

Dugassa GD, von Allen H, Schonbeck F (1996) Effect of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L. infected by fungal pathogen. Plant Soil 185:173–182

Dwivedi SK, Dwivedi P (2002) Mycorrhizae in ecosystems: an eco-friendly approach for improved plant growth. In: Rajak RC (ed) Biotechnology of microbes and sustainable utilization. Scientific Publishers, Jodhpur, pp 24–32

Eason WR, Scullion J, Scott EP (1999) Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes. Agric Ecosys Environ 73:245–255

Effmert U, Kalderas J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703

Elsen A, Baimey H, Swennen R, Waele DD (2003) Relative mycorrhizal dependency and mycorrhiza -nematode interaction in banana cultivars (Musa spp.) differing in nematode susceptibility. Plant Soil 256:303–313

Elsen A, Gervacio D, Swennen R, Waele DD (2008) AMF-induced biocontrol against plant parasitic nematodes in Musa sp. a systemic effect. Mycorrhiza 18:251–256

Facelli E, Smith SE, Facelli JM, Christophersen HM, Smith FA (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytol 185:1050–1061

Ferraz L, Brown D (2002) An introduction to nematodes-plant nematology. Pensoft, Sofia

Franzluebbers AJ, Wright SF, Stuedemann JA (2000) Soil aggregation and glomalin under pastures in the southern Piedmont, USA. Soil Sci Soc Am J 64:1018–1026

Fritz M, Jakobsen I, Lyngkjaer MF, Thordal-Christensen H, Pons- Kühnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16:413–419

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

Goicoechea N, Garmendia I, Sánchez-Díaz M, Aguirreolea J (2010) Review. Arbuscular mycorrhizal fungi (AMF) as bioprotector agents against wilt induced by *Verticillium spp*. in pepper. Span J Agric Res 8(S1):S25–S42

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Graham JH (2000) Assessing cost of arbuscular mycorrhizal symbiosis in agroecosystems. In: Podila GK, Douds DD Jr (eds) Current advances in mycorrhizal research. APS Press, St. Paul, NM, pp 127–140

Gworgwor NA, Weber HC (2003) Arbuscular mycorrhizal fungi–parasite–host interaction for the control of Striga hermonthica (Del.) Benth. in sorghum Sorghum bicolor (L.) Moench. Mycorrhiza 13:277–281

Hajra N, Shahina F, Firoza K (2013) Biocontrol of root-knot nematode by arbuscular mycorrhizal fungi in Luffa cylindrica. Pak J Nematol 31:77–84

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 Manage Sci 60:149–157

Hasan N, Jain RK (1987) Parasitic nematodes and vesicular-arbuscular mycorrhizal (VAM) fungi associated with berseem (*Trifolium alexandrinum* L.) in the Bundelkhan region. Indian J Nematol 17:184–188

Hawkins HJ, George E (2001) Reduced N15-nitrogen transport through arbuscular mycorrhizal hyphae to *Triticum aestivum* L. supplied with ammonium vs. nitrate nutrition. Ann Bot 87:303–311

Hearne SJ (2009) Control-the Striga conundrum. Pest Manage Sci 65:603–614

Hooker JE, Jaizme-Vega M, Alkinson D (1994) Biocontrol of plant pathogen using arbuscular mycorrhizal fungi. In: Gianinazzi S, Schhepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhauser, Basel, pp 191–209

Hussey RS, Roncadori RW (1982) Vesicular arbuscular mycorrhizal fungi may limit nematode activity and improve plant growth. Plant Dis 66:9–14

Jamal A, Ayub N, Usman M, Khan AG (2002) Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soyabean and lentil. Int J Phytoremed 4(3):205–221

John A, Bai H (2004) Evaluation of VAM for management of root knot nematodes in Brinjal. Indian J Nematol 34:22–25

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 fungus, *Glomus fasciculatum*. Indian J Nematol 35:32–36

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. Biores Technol 93:307–311

Koffi MC, Vos C, Draye X, Declerck S (2013) Effects of Rhizophagus irregularis MUCL 41833 on the reproduction of *Radopholus similis* in banana plantlets grown under in vitro culture conditions. Mycorrhiza 23:279–288

Lendzemo VW, Kuyper TW, Kropff MJ, van Ast A (2005) Field inoculation with arbuscular mycorrhizal fungi reduces Striga hermonthica performance on cereal crops and has the potential to contribute to integrated Striga management. Field Crop Res 91:51–61

Lendzemo VW, Van Ast A, Kuyper TW (2006) Can arbuscular mycorrhizal fungi contribute to Striga management on cereals in Africa? Outlook Agric 35:307–311

Lendzemo VW, Kuyper TW, Matusova R, Bouwmeester HJ, Ast AV (2007) Colonization by arbuscular mycorrhizal fungi of Sorghum leads to reduced germination and subsequent attachment and emergence of Striga hermonthica. Plant Sign Behav 2:58–62

Leta A, Selvaraj T (2013) Evaluation of arbuscular mycorrhizal fungi and Trichoderma species for the control of onion white rot (*Sclerotium cepivorum* Berk). J Plant Pathol Microbiol 4:1–6

Li AR, Smith SE, Smith FA, Guan KY (2012) Inoculation with arbuscular mycorrhizal fungi suppresses initiation of haustoria in the root hemiparasite *Pedicularis tricolor*. Ann Bot 109:1075–1080

Li AR, Guan KY, Stonor R, Smith SE, Smith FA (2013) Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root *hemiparasitic Pedicularis* species: do the fungal partners matter at low colonization levels? Ann Bot 112:1089–1098

Linderman RG (1994) Role of VAM fungi in biocontrol. In: Pfleger FL, Linderman RG (eds) Mycorrhizae and plant health. The American Phytopathological Society, St. Paul, MN, pp 1–27

Louarn J, Carbonne F, Delavault P, Becard G, Rochange S (2012) Reduced germination of *Orobanche cumana* seeds in the presence of arbuscular mycorrhizal fungi or their exudates. PLoS ONE 7(11):e49273. https://doi.org/10.1371/journal.pone.0049273

Marro N, Paola L, Cabello M, Doucet ME, Becerra AG (2014) Use of the arbuscular mycorrhizal fungus *Glomus intraradices* as biological control agent of the nematode *Nacobbus aberrans* parasitizing tomato. Braz Arch Biol Technol 57:668–674

Marschner P, Crowley D, Lieberei R (2001) Arbuscular mycorrhizal infection changes bacterial 16s DNA community composition in the rhizosphere of maize. Mycorrhiza 11:297–302

Merryweather J, Fitter A (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*: I. diversity of fungal taxa. New Phytol 138:117–129

Meyer JR, Linderman RG (1986) Selective influence on population of rhizospher or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Boil Biochem 18:191–196

Narayanan S (2005) Organic farming in India: relevance, problems and constraints. Occasional paper No. 38, Department of Economic Analysis and Research, National Bank for Agriculture and Rural Development, Mumbai

National Organic Standards Board (1997) United States department of agriculture national organic standards board. Definition of "Organic". United States Department of Agriculture, Washington, DC

Oehl F, Sieverding E, Mader P, Dubois D, Ineichen K, Boller T, Wiemken A (2004) Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138:574–583

Omolara OM (2014) Effects of mycorrhizal inoculant and organic mulches on nematode damage to cooking banana. J Biol Agric Health 19:81–86

Pandey RK, Goswami BK, Singh S (2004) Influence of soil pH on population dynamics of *Tylenhulus semipenetrans* infesting citrus and its biomanagement using AM fungi. Int J Nematol 14:174–176

Paulitz TC, Linderman RG (1991) Mycorrhizal interactions with soil organisms. In: Arora DK, Rai B, Mukerji KG, Knudsen GR (eds) Handbook of applied mycology: soil and plants. Marcel Dekker, New York, pp 77–129

Powell CL, Bagyaraj DJ (1984) VA Mycorrhiza. CRC Press, Boca Raton, FL. Schenek, NC

Pozo MJ, Azcón-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Raju PS, Clark RB, Ellis JR, Maranville JW (1990) Effects of species of VA-Mycorrhizal fungi on growth and mineral uptake of sorghum at different temperatures. Plant Soil 121:165–170

Ravnskov S, Jakobsen I (1995) Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytot 29:611–618

Reddy MS, Wang Q (2011) Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. Proceedings of the 2nd Asian PGPR conference August 21–24, Beijing, P.R. China

Rillig MC (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J Soil Sci 84:355–363

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

Rini VM (2001) Effect of arbuscular mycorrhiza on oil palm seedling growth and development of basal stem rot disease caused by *Ganoderma boninense*. Master thesis, Universiti Putra Malaysia

Rodriguez Romero AS, Jaizme-Vega MC (2005) Effect of the arbuscular mycorrhizal fungus Glomus manihotis on the root -knot nematode, *Meloidogyne javanica*, in banana. Nematol Medit 33:217–221

Ryan MH, Small DR, Ash JE (2000) Phosphorus controls the level of colonisation by arbuscular mycorrhizal fungi in conventional and biodynamic irrigated dairy pastures. Aust J Exp Agric 40:663–670

Sankaranarayanan C, Sundarababu R (1994) Interaction of *Glomus fasciculatum* with *Meloidogyne incognita* inoculated at different timings on blackgram (*Vigna mungo*). Nematol Medit 22:35–36

Scheublin TR, Van Logtestijn RSP, Van der Heijden MGA (2007) Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. J Ecol 95:631–638

Secilia J, Bagyaraj DJ (1987) Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Can J Microbiol 33:1069–1073

Sharma AK, Srivastava R (2008) Effect of transgenic crops in rhizosphere microorganisms: an overview. In: Biosafety issues related to practicing agriculture biotechnology. Department of Molecular Biology and Genetic Engineering, GBPUAT, Pantnagar, Vikrant Offset, Haldwani, pp 75–84

Shreenivasa R, Krishnappa K, Ravichandra NG (2007) Interaction effects of arbuscular mycorrhizal fungus *Glomus fasciculatum* and root-knot nematode, *Meloidogyne incognita* on growth and phosphorous uptake of tomato. Karnataka J Agric Sci 20:57–61

Siddiqui ZA, Akhtar MS (2007) Effects of AM fungi and organic fertilizers on the reproduction of the nematode *Meloidogyne incognita* and on the growth and water loss of tomato. Biol Fertil Soils 43:603–609

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 LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6:175–191
Singh S, Srivastava K, Sharma S, Sharma AK (2014) Mycorrhizal inoculum production. In: Solaiman ZM, Abbott LK, Varma A (eds) Mycorrhizal fungi: use in sustainable agriculture and forestry. Springer, Berlin, pp 67–79
Smith SE, Read DJ (1997a) Mycorrhizal symbiosis, 2nd edn. Academic Press, London
Smith SE, Read DJ (1997b) Vesicular-arbuscular mycorrhizas in agriculture and horticulture. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis, 2nd edn. Academic Press, London, pp 453–469
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London
Smith SE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20
Sui XL, Li AR, Chen Y, Guan KY, Zhuo L, Liu YY (2014) Arbuscular mycorrhizal fungi: potential biocontrol agents against the damaging root hemiparasite *Pedicularis kansuensis*. Mycorrhiza 24:187–195
Treeby MT (1992) The role of mycorrhizal fungi and non-mycorrhizal micro-organisms in iron nutrition of citrus. Soil Biol Biochem 24:857–864
Trotta A, Vanese GC, Gnavi EM, Fascon A, Sampo S, Berta G (1996) Interaction between the soil borne root pathogen *Phytophthora nicotianae* Var parasitica and the arbuscular mycorrhizal fungus Glomus mosseae in tomato plant. Plant Soil 185:199–209
Van der Heijden MGA (2010) Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91:1163–1171
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
Van der Heijden MGA, Rinaudo V, Verbruggen E, Scherrer C, Bàrberi P, Giovannetti M (2008) The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. 16th IFOAM Organic World Congress, Modena, Italy, June 16–20
Veresoglou SD, Rillig MC (2012) Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. Biol Lett 8:214–217
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
Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. New Phytol 172:554–562
Vos C, Claerhout S, Mkandawire 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 M, Tesfahun AN, Panis B, De Waele D, Elsen A (2012b) Arbuscular mycorrhizal fungi induce systemic resistance in tomato against the sedentary nematode Meloidogyne incognita and the migratory nematode Pratylenchus penetrans. Appl Soil Ecol 61:1–6
Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 1227:1198–1227
Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461
Wright SF, Anderson RL (2000) Aggregate stability and glomalin in alternative crop rotations for the central great plains. Biol Fertil Soil 31:249–253
Youssef MMA, El-Nagdi WMA (2015) Vesicular arbuscular mycorrhizae: a promising trend for biocontrolling plant parasitic nematodes. A review. Sci Agric 11:76–80

Zadehbagheri M, Azarpanah A, Javanmardi S (2014a) Perspective of arbuscular mycorrhizal fungi phytoremediation on contamination and remediation heavy metals soil in sustainable agriculture. Am Eurasian J Agric Environ Sci 14:379–386

Zadehbagheri M, Javanmardi S, Azarpanah A (2014b) Bioefficacy and characterization effect of arbuscular mycorrhizae fungi on defence response diseases and soil sickness in crop plants (review). Am Eurasian J Agric Environ Sci 14:363–378

Zhu YG, Christie P, Laidlaw AS (2001) Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. Chemosphere 42:193–199

Zimmermann J, Musyoki MK, Cadisch G, Rasche F (2016) Biocontrol agent *Fusarium oxysporum* f. sp. strigae has no adverse effect on indigenous total fungal communities and specific AMF taxa in contrasting maize rhizospheres. Fungal Ecol 23:1–10

Chapter 11
The Impact of AMF Symbiosis in Alleviating Drought Tolerance in Field Crops

Jaagriti Tyagi, Eram Sultan, Annu Mishra, Maya Kumari, and Ramesh Namdeo Pudake

Abstract Recent climate changes are expected to cause more frequent and severe drought affecting major field crops. Most of the cultivated field crops have a symbiotic association with the arbuscular mycorrhizal fungi (AMF) which is present in rhizosphere of these crops. Members of these class of fungi includes species of *Glomus*, *Gigaspora,* etc. have been found to be colonizing roots and forming an association with field crops for mutual benefits of both the partners. This symbiosis is known to help the plant to tolerate drought with the positive effects on plant growth. This chapter provides an overview of possible biochemical and genetic mechanism involved in AMF assisted drought tolerance in field crops. The improved water and nutrient absorption with the help of extraradical hyphal growth of AMF is one the important factor in helping plants to avoid the ill effects of drought. Along with this, by increased concentration of many biomolecules like amino acids, polyamines, hormones ; osmotic adjustment with the help of total soluble sugar (TSS), proline, ascorbic acid, and removing reactive oxygen species through antioxidant enzymes and antioxidants; AMF helps plant to reduce the effects of drought. Besides this, the results of some studies have given new exciting genetic perspectives including cellular water transport by mycorrhized roots.

J. Tyagi
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Noida, UP 201313, India

E. Sultan
Laboratory of Plant Immunity, Department of Life Science, NIT Rourkela, Rourkela, Odisha 769008, India

A. Mishra • R.N. Pudake (✉)
Amity Institute of Nanotechnology, Amity University Uttar Pradesh, Noida, UP 201313, India
e-mail: rnpudake@amity.edu

M. Kumari
Directorate General of Life Sciences, Defence Research and Development Organization, Rajaji Marg, New Delhi 110011, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_11

11.1 Introduction

Abiotic stresses are the major factors which directly and indirectly influence the plant development, productivity and cause huge yield losses worldwide (Zhu et al. 2012). Drought stress is the one of the major abiotic stress which is responsible for inducing the ill effects on plants like ionic and osmotic stress which results in oxidative stress. This oxidative stress causes the generation of reactive oxygen species (ROS) that are detrimental effects on plant cellular functions which needed for healthy cells (Nath et al. 2016). Increased ROS can damage the lipids, proteins and nuclear acids which can ultimately results in killing the cells (Rasool et al. 2013). Other physiological and biochemical processes affected by drought stress are photosynthesis, protein and energy synthesis, and metabolisms of lipid (Fig. 11.1). This scarcity of water also reduces the potential of soil water and decrease in uptake of nutrients ultimately reduces the biomass.

The majority of crop plants have been found to form association with rhizospheric fungi including AMF, and this symbiotic association is widely believed to protect host plants from the adverse effects of drought (Ruíz-Sánchez et al. 2011). All members of the Glomeromycota are obligate symbionts on plants, and are able to mobilize and transport of mineral nutrients (particularly phosphorus) from the soil which not accessible to roots (Corradi and Bonfante 2012). AMF produces highly branched fungal structures, arbuscules, within root cortical cells of their host plants, with which they exchange inorganic minerals, especially phosphorus and carbon compounds (Li et al. 2016b; Prasad et al. 2017). Plants are able to adapt in moisture deficient environment through two major strategies: drought avoidance and drought tolerance (Bray 1997). Mycorrhiza colonized plant may acts as drought tolerant as well as drought avoiders (Ruiz-Sánchez et al. 2010).

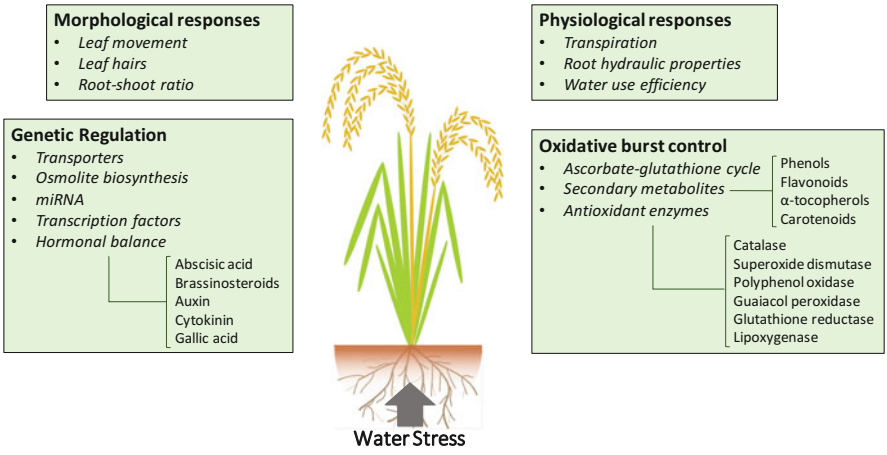


Fig. 11.1 Schematic representation of plants biochemical, physiological and genetic responses to water stress under normal growth condition

The positive role of AMF against various abiotic stresses has been recently reported by different authors (Evelin et al. 2009; Rapparini and Peñuelas 2014). It plays a multifactorial role by improving water and mineral nutrient uptake from surrounding soil by extensive extra-radical mycelium (ERM) (Fagbola et al. 2001; Smith et al. 2009; Goltapeh et al. 2008); and by inducing the accumulation of plant growth promoting hormones in hosts. It also improves soil properties by secretion of biomolecules; increase antioxidant enzyme activity in host (Garmendia et al. 2004); enhance the photosynthetic rate (Wu and Xia 2006); stomatal regulation and transpiration rate (Augé et al. 2015) as compared to non-mycorrhizal plants (Bárzana et al. 2012; Lee and Luan 2012). The aim of the present chapter is to outline the recent advances achieved in the study of drought tolerance by AM symbiosis with a particular focus on field crops. The probable mechanism that may be effective in improving the plant ability with the help of mycorrhiza to adapt themselves to drought conditions has been discussed.

11.2 Relation of Water and Nutrient Uptake Through AMF Hyphae and Plant's Tolerance to Drought

AMF have the ability to increase the surface area of roots by effectively producing intraradical and extraradical mycelium for better utilization and effective transportation of nutrition and water. ERM has great ability to mobilize and transform unusable inorganic minerals like phosphorus (P), nitrogen (N), zinc (Zn), or copper (Cu) into organic form which can be readily absorbed by host plants by arbuscules and vesicles (Smith and Read 2010). Findings on P and N uptake into plants *via* AMF, indicates the improvement in tolerance of plants exposed to extreme drought conditions (Subramanian et al. 1995; Smith et al. 2011). It has been reported that the comparative increase in green leaf area in AMF plants under drought stress occurs as a result of nitrogen acquisition by external hyphae that lead to more accumulation of protein in the leaves (Subramanian et al. 1995). The relative participation of the AMF pathway and plant root direct uptake pathways of P under water scarcity has not yet been estimated. But it is known that, AMF produces oxalic acid and phosphatase enzyme helping in solubilizing considerable amounts of phosphorus. Besides, this the micronutrient especially Zn and Cu uptake was also reported to be increased by mycorrhizal association (Suri et al. 2011).

This extensive extraradical hyphal network develops an interconnection between plant and soil. It is able to enter easily into the fine pores of soil, and has been reported that as low as 4% of water in hyphal compartment was transferred to the root compartment by ERM due to the presence of these highly specialized transportation network. AMF hyphal tips are hydrophilic in nature thus facilitate the water movement from the soil to the hyphal tip and ultimately transport it inside the plant cells. This extensive mycorrhiza network avoids the water depletion zone surrounding the roots (Miransari 2011; Smith et al. 2011).

It has been also reported that, AMF symbiosis improved plant fitness under water stress mainly by improving the plant water status and N uptake. Together it

improves the activities of N-assimilating enzymes like Glutamine synthetase, and resulted in increased amounts of proteins and amino acids (Ruiz-Lozano et al. 1996). The role of AMF hyphae in water uptake in water limiting conditions, as with P uptake, is still a matter of debate (Augé 2001; Smith et al. 2011). To resolve this dispute, an experimental study was conducted on barley plants inoculated with *Glomus intraradices* in a compartmented 'split plant-hyphal' chamber with a specifically adapted online system for monitoring the soil water content. They concluded that fungal hypha comprises 20% of the total water uptake of the plant. These findings were similar with earlier results which suggested a direct uptake and transfer water to the host plants *via* AMF hyphae (Khalvati et al. 2005). Along with improved water and nutrient uptake in plants, it may also affect root hydraulic conductivity in drought stress conditions.

11.3 Biochemical Responses of AMF Plants to Drought

Osmotic adjustment is one of the most important feature adapted by plants to resist drought stress, this involves accumulation of solutes and thus a decreased osmotic potential as well as increased pressure potential (Martinez et al. 2007). Osmotic adjustment helps the plants in maintaining a high relative water content (RWC) at a low leaf water potential, thereby supporting the plant growth (Farooq et al. 2009). It has been reported that AMF colonization improved the osmotic balance by accumulation of osmotic solutes and ions such as proline, non-structural carbohydrates, ionic accumulation (K^+, Ca^{2+} and Mg^{2+}) (Ruiz-Lozano 2003), resulting in the drought tolerance. Several recognized processes (e.g. ABA and proline accumulation) have been proposed to assist AMF inoculated plants to better alleviate drought stress. Therefore, mycorrhiza actually enhances the water uptake ability and hence leaf conductivity, turgor potential regulates osmotic adjustment by keeping stomata open longer to fix carbon more efficiently (Wu et al. 2013; Wu and Xia 2006). Under drought conditions, stomata remains close (stomatal resistance) in order to prevent the leaf-water status from dropping to critical levels (Bago et al. 2003). As reported earlier that AMF regulate the stomatal conductance and other physiological mechanism by triggering the ABA response (Ludwig-Müller 2010) (Fig. 11.2).

11.3.1 Role of Metabolites in Drought Tolerance of AMF Plants

11.3.1.1 Amino Acids

It has been found that many amino acids accumulate in plants exposed to various abiotic stresses. Proline is one of the most widely distributed osmolytes, and the level of which is raised in different abiotic stresses including drought, salinity and

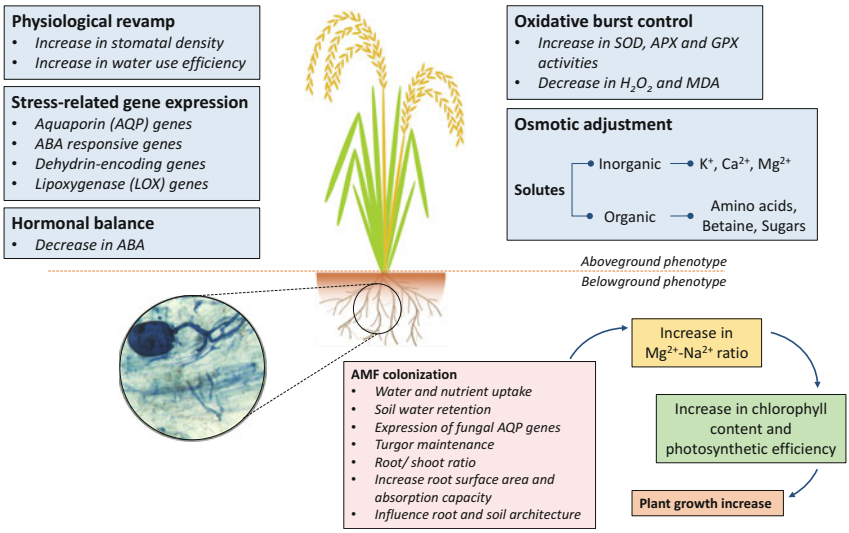


Fig. 11.2 Schematic representation of the role of AMF symbiosis in plant water stress. AMF help host plants to reduce the detrimental effects of water scarcity through direct or indirect ways by affecting plant physiological and biochemical processes in both roots and shoots

cold stress (Szabados and Savoure 2010). The relationship between the accumulation of osmolytes and stress tolerance has been discussed because of its role in plant growth promotion and increment of crop yield (Blum 2016). Similar observations were reported in maize, groundnut, coriander, soybean with AMF which develops drought tolerance by increasing the accumulation of some functional amino acids (Table 11.1).

11.3.1.2 Polyamines

Polyamines are low-molecular-weight cationic nitrogen compounds that interact with negatively charged molecules like nucleic acids, proteins and phospholipids. Due to their cationic nature, these commonly occurring compounds have been frequently related with environmental stresses, including drought, salinity and chilling stress, as well as UV-B and heavy metals (Groppa and Benavides 2008; Hussain et al. 2011). Under abiotic stress, polyamines have been attributed to be involved in the stabilization of membranes protecting them from denaturation, scavenging free radicals, modulating nucleic acid structures and also enzyme activities or function (Hasanuzzaman et al. 2013). Citrus plant showed that endogenous PAs regulate mycorrhizal development by altering the distribution of carbohydrates to roots (Wu et al. 2013). Various reports suggested that drought in mycorrhized plants increase in polyamine under water deficient, but it is yet not clear how endogenous PAs are transferred from AMF to the host plants.

Table 11.1 Biochemical and physiological responses of AM on crop plants during drought

Sr. No	Plant	Mycorhizza species	Biochemical and physiological characters studied	References
1	Bambara groundnut (*Vigna subterranea*)	*Glomus intraradices*, *Gigaspora gregaria*, *Scutellospora gregaria*	Mineral content, soluble sugars and acid phosphatise was increased with stress in presence to AMF, but proline was decreased	Tsoata et al. (2015)
2	*Barley (Hordeum vulgare)*	*G. intraradices*	Root volume, Phosphorus content, and phosphatase enzymes activity was increased, indicating that AMF not only regulated plant water relations, but also increases P acquisition and host growth in drought	Bayani et al. (2015)
		Rhizophagus intraradices	Phosphorus (P) concentration, leaf water potential, photosynthetic rate, transpiration rate, stomatal conductance, and WUE	Li et al. (2014)
3	Sweet potato (*Ipomoea batatas*)	*Glomus* sp.	Found that free proline and soluble sugars play a key role in water stressed plants with AMF symbiosis by adjusting osmotic potential	Yooyongwech et al. (2016)
4	Black locust (*Robinia pseudoacacia*)	*Funneliformis mosseae* and *R. intraradices*	Plant growth, LWC, chlorophyll concentration, photosynthesis, nutrient concentration, and fractal dimension (FD), root colonization percentage	Yang et al. (2014)
5	Chickpea (*Cicer arietinum*)	*G. intraradices, G. etunicatum* and *G. versiform*	Chlorophyll content, proline, antioxidant enzyme (POD, CAT, APX, PPO), MDA content	Sohrabi et al. (2012)
6	Common bean (*Phaseolus vulgaris*)	*G. mosseae* or *G. intraradices*	Plant growth parameters, Symbiosis development, RWC, Shoot nutrient contents	Franzini et al. (2010)
7	Common bean *(Phaseolus vulgaris*) and Maize (*Zea mays*)	*G. mosseae* and *G. intraradices*	RWC, mycorrhizal root colonization, N, P and K content, Total dry weight (g), Pod dry weight, Root dry weight, Nodules (weight, number), Stomatal conductance	Franzini et al. (2013)

(continued)

Table 11.1 (continued)

Sr. No	Plant	Mycorhizza species	Biochemical and physiological characters studied	References
8	Coriander *(Coriandrum sativum)*	*G. hoi*	Highest WUE and proline accumulation were achieved under stress conditions with AMF	Farahani et al. (2013)
9	Durum wheat (*Triticum aestivum durum*)	*R. intraradices*	Biomass of grains, and higher contents of copper, iron, manganese, zinc and gliadins in grains was not affected in drought in presence of AMF	Goicoechea et al. (2016)
10	Foxtail millet *(Setaria italica L.)*	*G. intraradices*	Greater plant height, collar diameter, panicle height, panicle weight and grain weight, higher enzyme activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR), and lower concentration of malondialdehyde (MDA) , H_2O_2, and $O2^-$	Gong et al. (2015)
11	Hardy sugarcane (*Saccharum arundinaceum*)	*Glomus* Spp.	Biomass, SWC, RWC, Root colonization percentage, osmotic potential, proline, TSS, protein, ascorbate, phenol, MDA, Glutathione, antioxidant enzymes (GPOX, SOD, APX), Chlorophyll flourescens, photosystem I and II activities	Mirshad and Puthur (2016)
12	Lentil (*Lens culinaris L.*)	*G. intraradices* and *G. mosseae*	Increased shoot dry weight, and grain protein with AMF	Aghayari et al. (2014)
13	Lettuce (*Lactuca sativa*)	*G. intraradices* and *G. mosseae*	RWC, starch, TSS, soluble proteins, secondary metabolites (carotenoids anthocyanins, chlorophylls and phenolics, anthocyanins), biomass	Baslam and Goicoechea (2012)
		G. intraradices or *G. mosseae*	Antioxidant enzyme activities (SOD, CAT, and POD), phosphatase and nitrate reductase activities, Solute accumulation	Kohler et al. (2008)

(continued)

Table 11.1 (continued)

Sr. No	Plant	Mycorhizza species	Biochemical and physiological characters studied	References
14	*Maize (Zea mays)* and Tomato (*Solanum lycopersicum)*	*G. intraradices*	Symbiotic development, Leaf water potential, RWC, Stomatal conductance, Photosynthetic efficiency, Hydrostatic root hydraulic conductivity, Relative apoplastic water flow	Bárzana et al. (2012)
15	Maize (*Zea mays*)	*G. mosseae*	Lipid peroxidation, antioxidant enzyme activity, isoenzyme expression.	George and Shaaban (2015)
		G. mossea, *G. intraradices*, *G. etunicatum*	Has positive effects on grain yield, and nutrient concentration	Ghorbanian et al. (2011)
		G. intraradices	Led to improve in WUE, SWC, Grain yield, biological yield	Naghashzadeh et al. (2015)
		G. intraradces	During moderate drought stress AMF significantly improved in quality and yield of maize	Sajedi et al. (2010)
		G. etunicatum	Root colonization percentage and plant biomass, Lipid peroxidation and membrane permeability, Proline content, antioxidant enzyme activity (SOD, CAT, and POD)	Zhu et al. (2011)
		G. etunicatum	Root, shoot and total dry weights, Gas exchange parameters including Pn, E, gs, and intercellular CO_2 concentration, chlorophyll, Chlorophyll fluorescence, RWC, WUE	Zhu et al. (2012)
16	Mung bean *(Vigna radiata*)	*G. mosseae*, *G. intraradices*	Seed and protein yield, WUE, leaf P, leaf N, harvest index of protein, and WUE	Habibzadeh et al. (2013)
17	Pigeon pea *(Cajanus cajan)*	*G. mosseae*	Physiological parameters, Chl content, photosynthetic rate, stomatal conductance, TSS, MDA, proline, root oxidase activity	Qiao et al. (2011)

(continued)

Table 11.1 (continued)

Sr. No	Plant	Mycorhizza species	Biochemical and physiological characters studied	References
18	Rice (*Oryza sativa)*	–	Shoot fresh weight, photosynthetic efficiency, glutathione, H_2O_2, MDA content	Ruiz-Sánchez et al. (2010)
		G. intraradices	Shoot water potential, Photosynthetic efficiency, Stomatal conductance, MDA content, proline content, glutathione and ascorbate contents, Biomass production.	Ruíz-Sánchez et al. (2011)
19	Safflower (Carthamus tinctorius)	*G. hoi*	Chlorophyll content, root and shoot length, shoot and root dry and fresh weight, grain yield	Shariati et al. (2015)
20	Saffron (*Crocus sativus*)	*G. aggregatum*, *G. mosseae*, *G. etunicatum*	AMF colonization percentage, plant growth parameters, carotenoid and chlorophyll, TSS, mineral content	Mohebi-Anabat et al. (2015)
21	Sorghum (*S. bicolor* L.)	*G. intraradices*	Grain yield and its components, Harvest index, root colonization percentage	Afshar et al. (2014)
22	Sorghum (*Sorghum bicolor*) and Pumpkin *(Cucurbita pepo)*	*G. intraradices* and *Gigaspoa margarita*	Significantly influenced stomatal conductance	Augé et al. (2007)
23	*Soybean (Glycine max)*	*Septoglomus constrictum*, *Glomus* sp., *G. aggregatum*	Water content, P and N concentrations, MDA, soluble sugars, chlorophyll content, proline	Grümberg et al. (2015)
24	Tomato (*Solanum lycopersicum)*	*R. irregularis*	Photosynthetic capacity, osmolyte accumulation, root hydraulic conductivity or aquaporin abundance and phosphorylation status	Calvo-Polanco et al. (2016)
		Funneliformis mosseae and *R. intraradices*	Plant biomass and AM root colonization, leaf water potential (Ψleaf) and gas exchanges (gs), chlorophyll content index, Stomatal density, ABA content, Proline and H_2O_2 content, and SOD activity	Chitarra et al. (2016a)

(continued)

Table 11.1 (continued)

Sr. No	Plant	Mycorhizza species	Biochemical and physiological characters studied	References
25	Tomato (*Lycopersicon esculatum*) and Bell Pepper (*Capsicum annuum*)	*R. intraradices*, and *R. fasciculatum*	Biomass, root length, shoot length and chlorophyll content, proline, CAT, GPOX activity	Tallapragada et al. (2016)
26	Watermelon (*Citrullus lanatus*)	*Glomus* sp. and *Paraglomus* sp.	Fruit yield, WUE, root-N and -P content ,	Omirou et al. (2013)
27	Wheat (*Triticum aestivum*)	*Glomus* spp.	Plant height, leaf area and fresh, dry matter yield, shoot water content, nutrients (N, P, K, Mg and Ca), photosynthetic pigments, mycorrhizal colonization percentage	Abo-Ghalia and Khalafallah (2008)
		G. mosseae or *G. etunicatum*	Mycorrhizal colonization percentage , Biomass and grain yields, nutrient uptake	Al-Karaki et al. (2004)
		G. claroideum	Biomass production, RWC, leakage of solutes, leaf chlorophyll and protein concentrations,	Beltrano and Ronco (2008)
		G. mosseae	RWC, osmotic potential, EL, chlorophyll content and fluorescence, ROS and MDA content, antioxidant enzymes (SOD, CAT, POD, APOX, Glutathione reductase), ascorbic acid, glutathione, proline, Enzymes of N and P metabolism, N, P, K content	Rani (2016)
		G. intraradices	Specific leaf area (SLA), relative water content, WUE, P and N uptake, plant biomass and grain yield	Zhang et al. (2013a)
		G. intraradices, G. mosseae, G. geosporum, G. claroideum	Photosynthesis, transpiration and stomatal conductance	Zhou et al. (2015)

11.3.1.3 Carbohydrates

It is well known that water stress lead to accumulation of the non-structural carbohydrates among many plant species (Bartels and Sunkar 2005). Soluble sugars play a dual role in the detoxification of ROS, and can either be involved in ROS producing metabolic pathways, and also regulate NADPH-producing metabolic pathway such as the oxidative pentose-phosphate (OPP) pathway and thereby contribute to ROS scavenging. Soluble carbohydrates generated by photosynthesis performs as osmoprotectants and signaling molecules under stressful environment (Baslam and Goicoechea 2012), and stabilizes cellular membranes and maintain turgor pressure and protecting the plant cell from degradation (Grümberg et al. 2015). Similar reports have found in bambara groundnut, lettuce, saffron (Table 11.1).

11.3.2 Protection of Host from Oxidative Damage by AMF Under Drought

11.3.2.1 Reduction of Oxidative Stress

ROS are partially oxidized free radicals, produced in all the aerobic organisms as a by-product of metabolism. In green plants, chloroplast and mitochondria is the most favourite site for ROS generation, as O_2 is continuously generated through water autolysis and readily available inside the organelle (Garg and Manchanda 2009). ROS includes both oxygen radicals, like superoxide (O_2^-), hydroxyl (^-OH), peroxyl (ROO^-), etc., and other non-radicals such as hydrogen peroxide (H_2O_2), singlet Oxygen etc. Under minimal concentration they acts as signaling molecules and involved in the activation of defense responses and in acclimation to unfavourable environmental conditions, while increased accumulation causes oxidative damage/burst (Suzuki et al. 2012; Nath et al. 2016). Plants developed various antioxidant mechanisms to maintain this delicate equilibrium by enzymatic and non-enzymatic systems present in plant cell organelles, which very often form a common metabolic pathway. Studies in chickpea, foxtail millet, maize and rice with AMF under drought stress have found similar results (Table. 11.1).

11.3.2.2 Role of Antioxidant Enzymes- SOD, CAT, POX, Ascorbate- POX in Drought Tolerance

Superoxide dismutase (SOD) is one of the most efficient antioxidant enzymes, which initiates the detoxification of free radicals. It catalyses the removal of $O2^-$ by its dismutation to H_2O_2 and O_2 (Koc and Ustun 2008), are found in various locations within the cell. It has been found that drought enhances its activity, and similar observation was reported in AMF inoculated maize, rice and other field

crops (Table 11.1). Catalase (CAT) found in the peroxisomes and further dismutase H_2O_2 to H_2O and O_2. It is one of the key defending enzymes for the protection of photosynthetic apparatus, and hence inhibits the autolysis of chloroplast under unfavourable environment (Xia et al. 2015). AMF colonization decreased the H_2O_2 content in wheat, tomato, bell pepper and rice under water scarcity (Table 11.1).

Guaicol peroxidases (GPOX) are a diverse group of enzymes which detoxify H_2O_2 in cytocol. Increased activity of GPOX was observed in many drought exposed AMF inoculated plants. It has been found that, there is an increased activity of POX enzyme in drought stressed foxtail millet (Gong et al. 2015) when have association with AMF.

Glutathione reductase plays a significant role in Ascorbate-glutathione cycle by converting the glutathione back into reduced state (Tausz et al. 2004). These enzymes primarily acts as scavenger for oxidized substances and also participates in stabilization of structural proteins by modification of (-SH) sulphur groups. It was reported in AM inoculated soybean plants, that increased activity of glutathione reductase lowers the glutathione level and hence lowers the oxidative tension inside the membrane (Porcel et al. 2007).

Ascorbate peroxidase enzyme abundantly found in chloroplast of mesophyll cells. It has been reported that H_2O_2 detoxification is carried out by ascorbate-glutathione cycle. The enzyme acts on H_2O_2 and breakdown it into H_2O and O_2 (Mittler 2002; Tyagi et al. 2017). Both the enzymes catalase and ascorbate peroxidise participates in detoxification of H_2O_2, but former involved in quenching of ROS and later participates in ROS signaling. Drought assisted enhancement in APOX activity was observed in many mycorrhiza colonized plants, such as soybean (Porcel et al. 2003).

Lipoxygenase are a group of enzymes which indirectly participates in defending action, by detoxification of hydroxyl peroxides and triggers the accumulation of malondialdehyde content. It is found to be involved in both—biotic and abiotic stress (Lim et al. 2015). Recent report indicates its defensive role in tomato plants (Cervantes-Gámez et al. 2016) (Table 11.1).

11.3.2.3 Role of Antioxidant Metabolite in AMF Assisted Drought Tolerance

Antioxidant metabolite machinery induces the accumulation of secondary metabolites such as phenols, flavonoids, carotenoids, α-tocopherols, and ascorbate–glutathione. These metabolites participate in the ROS detoxification and prevent the oxidative stress inside the plant cell. As the stress progresses ROS burst generates oxidative stress and plant inversely activates the antioxidant machinery (Mittler 2002). It has been studied by various researchers that AM colonization alleviate the ROS mediated oxidative burst by triggering the antioxidant machinery inside their host plant cell (Abbaspour et al. 2012; Baslam and Goicoechea 2012).

AM plants were reported to show diverse antioxidant and also cellular redox status (Rapparini and Peñuelas 2014).

Ascorbic acid is the powerful natural scavenger of OH^- that lowers the accumulations of MDA in cell membrane thus stabilizes the membrane integrity (Foyer and Noctor 2005). Ascorbic acid minimizes the oxidative stress through cooperation with other antioxidant molecules. It has reported that the increment in ascorbate content in AM citrus plant (Wu et al. 2010), and similar reports was observed in chickpea, hardy sugarcane, rice, and foxtail millet under water scare condition (Table 11.1).

Glutathione is the low molecular weight antioxidant compounds and actively participates in the various metabolic functions of plant cell (Tausz et al. 2004). Mycorrhization-mediated rises in photosynthetic performance under water deficiency was correlated with the accumulation of the antioxidant compounds such as glutathione, which in turn lowered cellular H_2O_2 and decreased membrane lipids (Ruíz-Sánchez et al. 2011). Similar results have found in wheat where *G. mossae* confers drought tolerance by increment of glutathione level (Rani 2016).

Phenol and flavonoids was extensively reported to play a role in alleviating drought stress, and was attributed as one of the ROS scavengers in plants. The AM inoculated host plant also get protected against oxidative damage (Abbaspour et al. 2012) by induced accumulation of secondary metabolites in wheat, rice under drought (Table 11.1).

11.4 Improvement in Soil Moisture Holding Capacity by AMF

The fertility of soil is greatly depends on its compactness which influences the agronomic yield. Since the mechanical and chemical methods are not very efficient and economical, use of biological methods to alleviate the soil fertility is recommended these days. It has been reported that AM fungal hyphae can enhance soil architecture due to their extensive filamentous hyphal network; also modifies the soil structure by forming aggregates through the production of the glycoprotein glomalin (Miransari et al. 2007; Singh et al. 2013). So AMF symbiosis may also able to increase drought resistance in plants through the improvement of soil structural stability that in turn increases the water holding capacity of soil and regulates the transport of nutrients, water flow and limit the loss of hydraulic conductivity caused by air pores (Augé 2001; Ruiz-Lozano 2003). It has been reported that mycorrhiza infected soil form more stable aggregates and significantly higher extraradical hyphal mycelium than the soils of non-inoculated plants. Glomalin-related soil protein (GRSP) which prevents the loss of water from the soil exposed to various abiotic stresses (Nichols 2008; Wu et al. 2014), thereby, regulating the water relations within soil–plant continuum. Glomalin also contains 30–40% carbon containing compounds that protect the soil from desiccation by

enhance the water holding capacity of soil (Sharma et al. 2017). Thus mycorrhiza can be considered as the best biological tool for improving soil structure. It has been hypothesized that hyphal network produces sticky material that causes soil particles to adhere and form aggregates. It has been proposed that AMF symbiosis increase the activity of soil phosphatase and hence enhancing tolerance of trifoliate orange under drought stress (Wu et al. 2013).

11.5 Physiological Response of AMF to Drought Stress

AMF symbiosis regulates the physiological functions such as leaf water potential (LWP), relative water content (RWC), stomatal conductance, photosynthesis II efficiency and CO_2 assimilation. AMF helps in amelioration of water stress by physiological modification of the above ground parts of the host plant (Augé 2001; Bárzana et al. 2012). Many experimental evidences have shown an increased the rates of gas exchange (stomatal conductance, transpiration, and photosynthetic rates) in mycorrhiza inoculated plants as compared to non-mycorrhizal plants under water limited conditions (Augé 2001; Ruiz-Lozano 2003; Khalvati et al. 2005; Lee and Luan 2012). Scarcity of water tends to decrease the stomatal conductance and increased diffusive resistance to CO_2 due to inhibition of RUBISCO activity, which could lead to increased plant water potential. To maintain water uptake from the soil, though, the water potential must be reduced. To maintain the turgor pressure in plant cell, plants can rely on mechanisms of 'osmotic adjustment' or 'osmoregulation' that decrease the osmotic potential. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the physiological activity of plants (Serraj and Sinclair 2002). It has been suggested that AMF mediate ABA response to triggers stomatal conductance and other physiological processes (Ludwig-Müller 2010).

11.5.1 Plant Gas Exchange

AMF have their direct impact in mitigating of water stress in host plant by regulating the gaseous exchange and maintain the hydration of leaf (Augé et al. 2001). However, the positive effect of AMF symbiosis in the regulation of leaf water potential as well as foliar gas exchange is still unclear. However, AMF colonized plant adopt these mechanism to avoid drought stress through maintain the leaf tissue under higher osmotic potential (Porcel and Ruiz-Lozano 2004; Bárzana et al. 2012; Bowles et al. 2016).

11.5.2 Role of Leaf Water Potential and Water Use Efficiency During Drought

Leaf water potential (LWP) is recognized as an index of the water status of an entire plant and hence represents an important feature revealing a potentially improved resistance of plants to drought through better hydration. Water-use efficiency (WUE) is defined as the ratio of biomass produced to the rate of transpiration. It was found that mycorrhizal plants imposed to water stress showed greater WUE than non-inoculated plants (Ruiz-Sánchez et al. 2010). WUE is important in crops growing in semiarid areas, because of high demand of water. Hence, its measurement provides an integrated assessment of plant water use and thus allows a further analysis of the plant–water relations of mycorrhizal plants when water is limiting. The symbiotic relationship between plant and AMF improves water uptake in arid conditions by improving the stomatal conductance and leaf turgor by effectively providing roots access to more of the soil water reservoir. As a result, AMF can protect host plants against drought conditions, and increase the plant growth under water scarcity. It has been shown that mycorrhizal plants can produce more roots than non-mycorrhizal plants under drought stress, which may lead to increased water transport (Li et al. 2014).

11.6 Plant Hormone Regulation by AMF During Drought

AMF have their positive influence on levels of jasmonate, terpenoids and carotenoids and phenols; which results in increase the activities of defence related enzymes like phenylalanine ammonialyase, polyphenol oxidase and peroxidase. In tomato plant AMF inoculation improved the phenolic acid profile in roots (López-Ráez et al. 2010). Several signaling molecules have been demonstrated to contribute to the phenolic synthesis, such as hydrogen peroxide (H_2O_2), salicylic acid (SA), and nitric oxide (NO) (Gayoso et al. 2010). AMF symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity under water stress in *Phaseolus vulgaris* plant (Sánchez-Romera et al. 2016). AMF colonized roots have higher jasmonic acid (JA) contents than control plants (Meixner et al. 2005). The ABA played an important role in stomatal regulation and has been suggested as one of the non-nutritional feature of AMF symbiosis under water scarcity. Several reports suggested that that ABA is increased in colonized plant facing the drought stress (Doubková et al. 2013). The signalling molecules like SA, H_2O_2, and NO were monitored and found that they could be involved in the local induction of phenolic biosynthesis by AMF in *Trifolium repense* (Zhang et al. 2013b).

11.7 Genes Involved During AMF and Field Crops Symbiosis During Drought

The physiological and biochemical responses of plant to drought stress with association with AMF are the results of expression of abiotic stress associated genes. The genes which are involved in signaling and regulatory pathways plays very important role during this association. The recent molecular studies have revealed that the membrane transport proteins- like aquaporin and phosphate transporters (Pudake et al. 2017) play important role in symbiotic association with AMF. Aquaporins are the class of membrane proteins which channels water, uncharged molecules in roots and leaves cells (Conner et al. 2013). These genes have been found to increases root hydraulic conductivity in roots and leaf water potential and decreased transpiration rates in rice and common bean plants with AMF association (Aroca et al. 2007; Ruíz-Sánchez et al. 2011). The regulation of aquaporins is mainly controlled by water scarcity, and may be involved in symbiotic exchange of water at plant-fungus interface. The AMF regulation of these proteins under drought stress has been reported to be involved in crop plant status and drought tolerance in other crops like *Medicago* (Uehlein et al. 2007) Lettuce (del Mar Alguacil et al. 2009) and maize (Bárzana et al. 2014). The fungal aquaporins are also found to be affected by drought stress (Li et al. 2013) and found to be contributing in maize plants tolerance to drought (Li et al. 2016b). A reduced expression of aquaporin genes in mycorrhized common bean plants has been reported when compared to nonmycorrhizal plants under the drought stress (Aroca et al. 2007), but it was postulated that the transport of other ions by these aquaporins might have important role in limited water condition. These studies in field crops and other studies in fruit crops support the potential water transport in host plants through AM, and helping the host plants in drought tolerance through aquaporin regulations (Table 11.2).

Recent findings suggest a potential involvement of the other genes were also involved during drought stress in mycorrhized crop plants. In one study with Pigeon pea inoculated with *G. Mosseea* found that 102 genes were upregulated and 40 genes were downregulated, and the putative functions of these genes were implicated in abiotic stress tolerance (Qiao et al. 2012). And in another study, genes involved in regulation of stomata development were promoted by a specific AM fungus (Chitarra et al. 2016a, b). A BiP-encoding gene from the AM fungus *G. intraradices* was up-regulated by drought stress in mycorrhizal plants, indicating that AM fungi contribute to better drought tolerance through proteins with chaperone-like activity (Porcel et al. 2007) (Table 11.2).

The genetic mechanisms of nutrient and water transfer between the AMF and plants during water scarcity are not well defined, so further studies with new technologies like transcriptome sequencing and proteomics should provide a better understanding of genes in enhanced drought resistance of AMF crop plants (Fig. 11.3).

Table 11.2 Genetic basis of water relations and other physiological processes involved in AMF Symbiosis under drought

Sr. No	Plant	Mycorrhiza Species	Genes studied	References
1	Carrot (*Daucus carota*)	*G. intraradices*	Two functional aquaporin genes- *GintAQPF1* and *GintAQPF2* were upregulated for improving plant water relations during the drought	Li et al. (2013)
2	Common bean (*P. vulgaris*)	*G. intraradices*	Aquaporin genes—*PvPIP1;2 PvPIP1;3* and *PvPIP2;1* responded differently to drought stress but was depended on the AMF presence	Aroca et al. (2007)
3	Lettuce (*L. sativa*)	*G. intraradices*	Enhanced expression of the PIP2 gene only in presence of AMF but not with plant-growth-promoting rhizobacterium (PGPR)	del Mar Alguacil et al. (2009)
4	Maize (*Z. mays*)	*R. intraradices*	A wide number of aquaporin subfamily genes were regulated by presence of AMF and different water level	Bárzana et al. (2014)
5	Maize (*Z. mays*)	*Rhizophagus intraradices*	Plant genes encoding D-myo-inositol-3-phosphate synthase (IPS) and 14-3-3-like protein GF14 (14-3GF), which were responsible for ABA signal transduction, was found to be involved in the activation of 14-3-3 protein and aquaporins (*GintAQPF1* and *GintAQPF2*) in *R. intraradices*	Li et al. (2016a)
6	Pigeon pea (*C. cajan*)	*G. mosseae*	120 upregulated and 40 downregulated genes that has putative functions implicated in abiotic stress tolerances	Qiao et al. (2012)
7	Soybean (*G. max*), lettuce (*L. sativa*), maize (*Z. mays*) and tobacco (*Nicotiana tabacum*)	*G. intraradices*	Binding immunoglobulin protein (BiP) encoding gene from the AM fungus was up-regulated by drought stress	Porcel et al. (2007)

(continued)

Table 11.2 (continued)

Sr. No	Plant	Mycorrhiza Species	Genes studied	References
8	Soybean (*G. max*), lettuce (*L. sativa*)	*G. mosseae*	Δ^1-pyrroline-5-carboxylate synthetase (p5cs) gene was upregulated in drought	Porcel et al. (2004)
9	Tomato (*Solanum lycopersicum*)	*F. mosseae* and *R. intraradices*	Expression study of LeEPFL9- STOMAGEN, and genes encoding EPF1 and EPF2, two intercellular signalling factors that function as antagonists of LeEPFL9, revealed that regulation of stomata development is promoted by a specific AM fungus	Chitarra et al. (2016b)

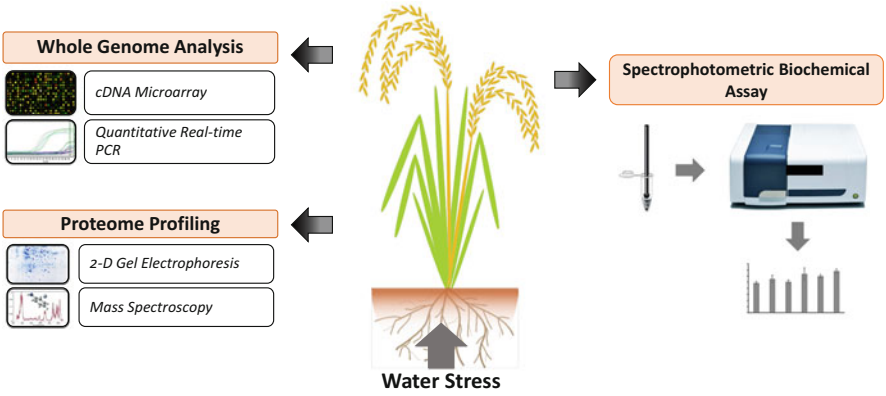


Fig. 11.3 Molecular and biochemical tools that can be used for evaluation of impact of mycorrhiza on plant growth during drought stress

11.8 Conclusion

The crop plants use various protective mechanisms to reduce the ill effects of water scarcity. Significant progress has been made in understanding the role of mycorrhizal symbiosis in alleviating drought tolerance, but there are still missing links for fully decoding its mechanism. Day by day there is an accumulation genetic, proteomics and metabolomics data recorded during the various biological processes in many crops; and this new 'omic' data will benefit in future to elucidate the mechanisms of AMF assisted drought avoidance and/or tolerance.

Acknowledgements RNP gratefully acknowledges the funding under Start-up Research Grant (Life Sciences) by Science and Engineering Research Board, Department of Science & Technology, Government of India (SB/FT/LS-104/2012).

References

Abbaspour H, Saeidi-Sar S, Afshari H, Abdel-Wahhab M (2012) Tolerance of mycorrhiza infected pistachio (*Pistacia vera* L.) seedling to drought stress under glasshouse conditions. J Plant Physiol 169:704–709

Abo-Ghalia HH, Khalafallah AA (2008) Responses of wheat plants associated with arbuscular mycorrhizal fungi to short-term water stress followed by recovery at three growth stages. J Appl Sci Res 4:570–580

Afshar RK, Jovini MA, Chaichi MR, Hashemi M (2014) Grain sorghum response to arbuscular mycorrhiza and phosphorus fertilizer under deficit irrigation. Agron J 106:1212–1218

Aghayari F, Maleki S, Ardakani MR, Rejali F, Faregh AH (2014) Growth and yield of lentil (*Lens culinaris* L.), as affected by mycorrhizal symbiosis and *Azospirillum brasilense* under rainfed conditions. Int J Biosci 4:253–262

Al-Karaki G, McMichael B, Zak J (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

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

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Augé RM, Stodola AJ, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97

Augé RM, Toler HD, Moore JL, Cho K, Saxton AM (2007) Comparing contributions of soil versus root colonization to variations in stomatal behavior and soil drying in mycorrhizal *Sorghum bicolor* and *Cucurbita pepo*. J Plant Physiol 164:1289–1299

Augé RM, Toler HD, Saxton AM (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13–24

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol 131:1496–1507

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Crit Rev Plant Sci 24:23–58

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Ruiz-Lozano JM (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

Bárzana G, Aroca R, Bienert GP, Chaumont F, Ruiz-Lozano JM (2014) New insights into the regulation of aquaporins by the arbuscular mycorrhizal symbiosis in maize plants under drought stress and possible implications for plant performance. Mol Plant Microbe Interact 27:349–363

Baslam M, Goicoechea N (2012) Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22:347–359

Bayani R, Saateyi A, Faghani E (2015) Influence of arbuscular mycorrhiza in phosphorus acquisition efficiency and drought-tolerance mechanisms in barley (*Hordeum vulgare* L.) Int J Biosci 7:86–94

Beltrano J, Ronco MG (2008) Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: effect on growth and cell membrane stability. Braz J Plant Physiol 20:29–37

Blum A (2016) Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ 40:4–10

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016) Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Sci Total Environ 566:1223–1234

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2:48–54

Calvo-Polanco M, Sánchez-Romera B, Aroca R, Asins MJ, Declerck S, Dodd IC, Martinez-Andujar C, Albacete A, Ruiz-Lozano JM (2016) Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. Environ Exp Bot 131:47–57

Cervantes-Gámez RG, Bueno-Ibarra MA, Cruz-Mendívil A, Calderón-Vázquez CL, Ramírez-Douriet CM, Maldonado-Mendoza IE, Villalobos-López MÁ, Valdez-Ortíz Á, López-Meyer M (2016) Arbuscular mycorrhizal symbiosis-induced expression changes in *Solanum lycopersicum* leaves revealed by RNA-seq analysis. Plant Mol Biol Rep 34:89–102

Chitarra W, Maserti B, Gambino G, Guerrieri E, Balestrini R (2016a) Arbuscular mycorrhizal symbiosis-mediated tomato tolerance to drought. Trends Plant Sci 11:1009–1023

Chitarra W, Pagliarani C, Maserti B, Lumini E, Siciliano I, Cascone P, Schubert A, Gambino G, Balestrini R, Guerrieri E (2016b) Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol 171:1009–1023

Conner AC, Bill RM, Conner MT (2013) An emerging consensus on aquaporin translocation as a regulatory mechanism. Mol Membr Biol 30:101–112

Corradi N, Bonfante P (2012) The arbuscular mycorrhizal symbiosis: origin and evolution of a beneficial plant infection. PLoS Pathog 8:e1002600

del Mar Alguacil M, Kohler J, Caravaca F, Roldán A (2009) Differential effects of *Pseudomonas mendocina* and *Glomus intraradices* on lettuce plants physiological response and aquaporin PIP2 gene expression under elevated atmospheric CO_2 and drought. Microb Ecol 58:942–951

Doubková P, Vlasáková E, Sudová R (2013) Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on *Knautia arvensis* plants in serpentine soil. Plant Soil 370:149–161

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Fagbola O, Osonubi O, Mulongoy K, Odunfa S (2001) Effects of drought stress and arbuscular mycorrhiza on the growth of *Gliricidia sepium* (Jacq). Walp, and *Leucaena leucocephala* (Lam.) de Wit. in simulated eroded soil conditions. Mycorrhiza 11:215–223

Farahani A, Lebaschi H, Hussein M, Hussein SA, Reza VA, Jahanfar D (2013) Effects of arbuscular mycorrhizal fungi, different levels of phosphorus and drought stress on water use efficiency, relative water content and proline accumulation rate of coriander (*Coriandrum sativum* L.) J Med Plants Res 2:125–131

Farooq M, Wahid A, Lee DJ (2009) Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiol Plant 31:937–945

Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28:1056–1071

Franzini VI, Azcón R, Mendes FL, Aroca R (2010) Interactions between *Glomus* species and *Rhizobium* strains affect the nutritional physiology of drought-stressed legume hosts. J Plant Physiol 167:614–619

Franzini VI, Azcón R, Méndes FL, Aroca R (2013) Different interaction among *Glomus* and *Rhizobium* species on *Phaseolus vulgaris* and *Zea mays* plant growth, physiology and symbiotic development under moderate drought stress conditions. J Plant Growth Regul 70:265–273

Garg N, Manchanda G (2009) ROS generation in plants: boon or bane? Plant Biosyst 143:81–96

Garmendia I, Goicoechea N, Aguirreolea J (2004) Effectiveness of three Glomus species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. Biol Control 31(3):296–305

Gayoso C, Pomar F, Novo-Uzal E, Merino F, de Ilárduya ÓM (2010) The Ve-mediated resistance response of the tomato to *Verticillium dahliae* involves H_2O_2, peroxidase and lignins and drives PAL gene expression. BMC Plant Biol 10:232

George NM, Shaaban LD (2015) Molecular and physiological changes in mycorrhizal *Zea mays* under different irrigation levels. Egypt J Exp Biol 11:1–9

Ghorbanian D, Harutyunyan S, Mazaheri D, Rejali F (2011) Effects of mycorrhizal symbiosis and different levels of phosphorus on yield, macro and micro elements of *Zea mays* L. under water stress condition. Afr J Agric Res 6:5481–5489

Goicoechea N, Bettoni M, Fuertes-Mendizábal T, González-Murua C, Aranjuelo I (2016) Durum wheat quality traits affected by mycorrhizal inoculation, water availability and atmospheric CO_2 concentration. Crop Pasture Sci 67:147–155

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, Heidelberg, pp 3–28

Gong M, You X, Zhang Q (2015) Effects of *Glomus intraradices* on the growth and reactive oxygen metabolism of foxtail millet under drought. Ann Microbiol 65:595–602

Groppa M, Benavides M (2008) Polyamines and abiotic stress: recent advances. Amino Acids 34:35

Grümberg BC, Urcelay C, Shroeder MA, Vargas-Gil S, Luna CM (2015) The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean. Biol Fertil Soils 51:1–10

Habibzadeh Y, Pirzad A, Zardashti MR, Jalilian J, Eini O (2013) Effects of arbuscular mycorrhizal fungi on seed and protein yield under water-deficit stress in mung bean. Agron J 105:79–84

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci 14:9643–9684

Hussain SS, Ali M, Ahmad M, Siddique KH (2011) Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. Biotechnol Adv 29:300–311

Khalvati M, Hu Y, Mozafar A, Schmidhalter U (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biol 7:706–712

Koc E, Ustun AS (2008) Defence against pathogen in plants and antioxidants. Erciyes Uni Sci Instit J 24:82–100

Kohler J, Hernández JA, Caravaca F, Roldán A (2008) Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35:141–151

Lee SC, Luan S (2012) ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant Cell Environ 35:53–60

Li T, Hu YJ, Hao ZP, Li H, Chen BD (2013) Aquaporin genes GintAQPF1 and GintAQPF2 from *Glomus intraradices* contribute to plant drought tolerance. Plant Signal Behav 8:e24030

Li T, Lin G, Zhang X, Chen Y, Zhang S, Chen B (2014) Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. Mycorrhiza 24:595–602

Li T, Sun Y, Ruan Y, Xu L, Hu Y, Hao Z, Zhang X, Li H, Wang Y, Yang L (2016a) Potential role of D-myo-inositol-3-phosphate synthase and 14-3-3 genes in the crosstalk between *Zea mays* and *Rhizophagus intraradices* under drought stress. Mycorrhiza 26:879–893

Li X, Zeng R, Liao H (2016b) Improving crop nutrient efficiency through root architecture modifications. J Integr Plant Biol 58:193–202

Lim CW, Han SW, Hwang IS, Kim DS, Hwang BK, Lee SC (2015) The pepper lipoxygenase CaLOX1 plays a role in osmotic, drought, and high salinity. Plant Cell Physiol 56:930–942

López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Exp Bot 61 (10):2589–2601

Ludwig-Müller J (2010) Hormonal responses in host plants triggered by arbuscular mycorrhizal fungi. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Dordrecht, pp 169–190

Martinez J, Silva H, Ledent J, Pinto M (2007) Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.) Eur J Agron 26:30–38

Meixner C, Ludwig-Müller J, Miersch O, Gresshoff P, Staehelin C, Vierheilig H (2005) Lack of mycorrhizal autoregulation and phytohormonal changes in the super nodulating soybean mutant nts1007. Planta 222:709–715

Miransari M (2011) Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. Biotechnol Adv 29:645–653

Miransari M, Bahrami H, Rejali F, Malakouti M, Torabi H (2007) Using arbuscular mycorrhiza to reduce the stressful effects of soil compaction on corn (*Zea mays* L.) growth. Soil Biol Biochem 39:2014–2026

Mirshad P, Puthur JT (2016) Arbuscular mycorrhizal association enhances drought tolerance potential of promising bioenergy grass (*Saccharum arundinaceum*). Environ Monit Assess 188:1–20

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mohebi-Anabat M, Riahi H, Zanganeh S, Sadeghnezhad E (2015) Effects of arbuscular mycorrhizal inoculation on the growth, photosynthetic pigments and soluble sugar of *Crocus sativus* (saffron) in autoclaved soil. Int J Agron Agric Res 6:296–304

Naghashzadeh M, Bour K, Pezeshkpour P (2015) Response of water use efficiency to mycorrhizal biofertilizer in maize under water stress conditions. Bull Env Pharmacol Life Sci 4:152–157

Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N (2016) Reactive oxygen species generation-scavenging and signaling during plantarbuscular mycorrhizal and Piriformospora indica interaction under stress condition. Front Plant Sci 7:1574

Nichols KA (2008) Indirect contributions of AM fungi and soil aggregation to plant growth and protection. In: Siddiqui ZA, Akhtar MS, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Berlin, pp 177–194

Omirou M, Ioannides IM, Ehaliotis C (2013) Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress only. Appl Soil Ecol 63:112–119

Porcel R, Barea JM, Ruiz-Lozano JM (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157:135–143

Porcel R, Azcón R, Ruiz-Lozano JM (2004) Evaluation of the role of genes encoding for Δ1-pyrroline-5-carboxylate synthetase (P5CS) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. Physiol Mol Plant Path 65:211–221

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, 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

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

Pudake RN, Mehta CM, Mohanta TK, Sharma S, Varma A, Sharma AK (2017) Cloning and expression analysis of four phosphate transporter genes (EcPT) from Eleusine coracana in response to mycorrhizal colonization and Pi stress. 3 Biotech 7:17

Qiao G, Wen X, Yu L, Ji X (2011) The enhancement of drought tolerance for pigeon pea inoculated by arbuscular mycorrhizae fungi. Plant Soil Environ 57:541–546

Qiao G, Wen X, Yu L, Ji X (2012) Identification of differentially expressed genes preferably related to drought response in pigeon pea (*Cajanus cajan*) inoculated by arbuscular mycorrhizae fungi (AMF). Acta Physiol Plant 34:1711–1721

Rani B (2016) Effect of arbuscular mycorrhiza fungi on biochemical parameters in wheat (Triticum aestivum L.) under drought conditions. Doctoral dissertation, CCSHAU, Hisar

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, New York, pp 21–42

Rasool S, Ahmad A, Siddiqi T, 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

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Ruiz-Lozano J, Azcon R, Gomez M (1996) Alleviation of salt stress by arbuscular mycorrhizal Glomus species in *Lactuca sativa* plants. Physiol Plant 98:767–772

Ruiz-Sánchez M, Aroca R, Muñoz Y, 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

Ruíz-Sánchez M, Armada E, Muñoz Y, de Salamone IEG, Aroca R, Ruíz-Lozano JM, Azcón R (2011) Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J Plant Physiol 168:1031–1037

Sajedi N, Ardakani M, Rejali F, Mohabbati F, Miransari M (2010) Yield and yield components of hybrid corn (*Zea mays* L.) as affected by mycorrhizal symbiosis and zinc sulfate under drought stress. Physiol Mol Biol Plants 16:343–351

Sánchez-Romera B, Ruiz-Lozano JM, Zamarreño ÁM, García-Mina JM, Aroca R (2016) Arbuscular mycorrhizal symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity caused by drought. Mycorrhiza 26:111–122

Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ 25:333–341

Shariati J, Weisany W, Torabian S (2015) Effect of azotobacter and arbuscular mycorrhizal on growth of safflower (*Carthamus tinctorius* L.) at different irrigation regimes. Biotechnol J 18:04

Sharma S, Prasad R, Varma A, Sharma AK (2017) Glycoprotein associated with *Funneliformis coronatum, Gigaspora margarita* and *Acaulospora scrobiculata* suppress the plant pathogens in vitro. Asian J Plant Pathol. https://doi.org/10.3923/ajppaj.2017

Singh PK, Singh M, Tripathi BN (2013) Glomalin: an arbuscular mycorrhizal fungal soil protein. Protoplasma 250:663–669

Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic Press, New York

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

Smith SE, Jakobsen I, Grønlund 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

Sohrabi Y, Heidari G, Weisany W, Golezani KG, Mohammadi K (2012) Changes of antioxidative enzymes, lipid peroxidation and chlorophyll content in chickpea types colonized by different Glomus species under drought stress. Symbiosis 56:5–18

Subramanian K, Charest C, Dwyer L, Hamilton R (1995) Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling. New Phytol 129:643–650

Suri V, Choudhary AK, Chander G, Verma T, Gupta M, Dutt N (2011) Improving phosphorus use through co-inoculation of vesicular arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria in maize in an acidic Alfisol. Commun Soil Sci Plant Anal 42:2265–2273

Suzuki N, Koussevitzky S, Mittler R, Miller G (2012) ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ 35:259–270

Szabados L, Savoure A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Tallapragada P, Dikshit R, Seshagiri S (2016) Influence of Rhizophagus spp. and burkholderia seminalis on the growth of tomato (Lycopersicon esculatum) and bell pepper (Capsicum annuum) under drought stress. Commun Soil Sci Plant Anal 47:1975–1984

Tausz M, Šircelj H, Grill D (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? J Exp Bot 55:1955–1962

Tsoata E, Njock SR, Youmbi E, Nwaga D (2015) Early effects of water stress on some biochemical and mineral parameters of mycorrhizal *Vigna subterranea* (L.) Verdc.(Fabaceae) cultivated in Cameroon. Int J Agron Agric Res 7:21–35

Tyagi J, Varma A, Pudake RN (2017) Evaluation of comparative effects of arbuscular mycorrhiza (Rhizophagus intraradices) and endophyte (Piriformospora indica) association with finger millet (Eleusine coracana) under drought stress. Eur J Soil Biol 81:1–10

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R (2007) Arbuscular mycorrhizal symbiosis and plant aquaporin expression. Phytochemistry 68:122–129

Wu QS, Xia RX (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425

Wu Q, Zou Y, Liu W, Ye X, Zai H, Zhao L (2010) Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. Plant Soil Environ 56:470–475

Wu QS, Srivastava A, Zou YN (2013) AMF-induced tolerance to drought stress in citrus: a review. Sci Hortic 164:77–87

Wu Z, McGrouther K, Huang J, Wu P, Wu W, Wang H (2014) Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: field experiment. Soil Biol Biochem 68:283–290

Xia XJ, Zhou YH, Shi K, Zhou J, Foyer CH, Yu JQ (2015) Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. J Exp Bot 66:2839–2856

Yang Y, Tang M, Sulpice R, Chen H, Tian S, Ban Y (2014) Arbuscular mycorrhizal fungi alter fractal dimension characteristics of *Robinia pseudoacacia* L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J Plant Growth Regul 33:612–625

Yooyongwech S, Samphumphuang T, Tisarum R, Theerawitaya C, Cha-um S (2016) Arbuscular mycorrhizal fungi (AMF) improved water deficit tolerance in two different sweet potato genotypes involves osmotic adjustments via soluble sugar and free proline. Sci Hortic 198:107–117

Zhang B, Liu W, Chang S, Anyia A (2013a) Phosphorus fertilization and fungal inoculations affected the physiology, phosphorus uptake and growth of spring wheat under rainfed conditions on the Canadian prairies. J Agron Crop Sci 199:85–93

Zhang RQ, Zhu HH, Zhao HQ, Yao Q (2013b) Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. J Plant Physiol 170:74–79

Zhou Q, Ravnskov S, Jiang D, Wollenweber B (2015) Changes in carbon and nitrogen allocation, growth and grain yield induced by arbuscular mycorrhizal fungi in wheat (*Triticum aestivum* L.) subjected to a period of water deficit. Plant Growth Regul 75:751–760

Zhu X, Song F, Liu S (2011) Arbuscular mycorrhiza impacts on drought stress of maize plants by lipid peroxidation, proline content and activity of antioxidant system. J Food Agric Environ 9:583–587

Zhu X, Song F, Liu S, Liu T, Zhou X (2012) Arbuscular mycorrhizae improves photosynthesis and water status of *Zea mays* L. under drought stress. Plant Soil Environ 58:186–191

Chapter 12
Arbuscular Mycorrhiza: A Tool for Enhancing Crop Production

Suvigya Sharma, Anil Kumar Sharma, Ram Prasad, and Ajit Varma

Abstract Soil microbes play a crucial role in determining many key components such as soil fertility, soil biodiversity and plant health. Due to excessive use of intensive agricultural practices like high inputs of pesticides, insecticides and inorganic fertilizers, the existence of these soil microbes having promising characteristics have become marginalized. However, in today's time introduction of environmental protection programs have created much awareness in many countries, including India, and intensive agricultural pattern is shifting towards low input (sustainable) agricultural regimes. Low input (sustainable) agriculture systems includes minimizing the use of mineral fertilizers, chemical pesticides and other such products and promoting organic and low cost methods into the agricultural system for better yield and protection against diseases. It is therefore, of vital importance for us to understand and manipulate the naturally occurring microorganisms for better crop productivity and establishment of sustainable agroecosystems. Characterization of beneficial soil microbes would be an important step towards understanding such below ground interactions. The focus of this chapter is upon understanding the functioning of Arbuscular mycorrhizae, its ecological significance and possible role in enhancing crop production.

S. Sharma (✉)
Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, India

Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 Fourth Floor, Noida 201313, India
e-mail: suvigya.sharma@gmail.com

A.K. Sharma
Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, India

R. Prasad • A. Varma
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3 Fourth Floor, Noida 201313, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_12

12.1 Introduction

Crop production all across the globe reached new heights with the onset of green revolution (between 1930s and late 1960s); which encouraged the development of new high-yielding varieties of cereal grains, modernization of irrigation infrastructure, emergence of technology management techniques, availability of hybridized seeds, chemical fertilizers and pesticides to farmers. Out of many beneficial effects of this change in agricultural sector and conventional farming practices, this cannot suffice the human population around the world, as the gap between the crop production and population kept on increasing which led to food shortage and food security issues in near future (Tilman et al. 2011). Heavy inputs of chemical fertilizers and pesticides have led to degradation of soil health and its biodiversity at all levels. Therefore, lower input sustainable agricultural practices are the need of the hour, which includes the replacement of conventional intensive agriculture regimes with cost effective planning and implementation and this can only be achieved by research based strategy of manipulating soil microbial communities and their interactions with in the agri-ecosystems and its rhizosphere. In this chapter Arbuscular Mycorrhiza fungi (AMF) and its role in crop production is discussed.

Decades of research on mycorrhizal fungi have proved that these are the most widespread and beneficial mycosymbionts and their symbiotic association were observed commonly with most of terrestrial plant families. Out of different types of these mycorrhizal fungi, the most commonly found are arbuscular mycorrhiza fungi. It is observed 70–90% of all land plant species form AM symbiosis (Brundrett 2009) AM fungi are obligate, bio-trophic and symbiotic in nature, they colonize the roots of most of the crop plants in natural and agricultural ecosystems (Leal et al. 2009). The infection zone of arbuscular mycorrhiza (AM) is on the surface of the plant root or inside the cortical cells, that is how they got their name—(ancient Greek: μυκης (mykes) = fungus and ριζα (rhiza) = root) (Frank and Trappe 2005). AM form arbuscules, which are fine and branched hyphae formed inside the cortical cells, AM hyphae extends outward from the root and helps in transport of inorganic nutrients such as P, N and micronutrients, water from the soil to the plant and collect carbon assimilate from the host plants for their survival (Bucher et al. 2009) also, AMF can strongly enhance the absorption rate of fertilizers, especially Phosphorous based (Tawaraya et al. 2012). AMF produce a polysaccharide compound known as glomalin through their external hyphal structures, which helps in improving the soil structure (Wright and Anderson 2000), also, they increase water availability to their host plant and helps in disease suppression (Graham 2001). AMF have positive impacts on plant growth promotion, soil reclamation and phyto-remediation (Leyval et al. 2002) with all these attributes, AMF can be used as a "Biofertilizer" in a sustainable agro ecosystem.

12.2 Plant Growth Promotion by Arbuscular Mycorrhizal Fungi

The AM fungi makes most dominant component of the rhizosphere, it provides plants with nutrient uptake and many other beneficial inputs for its plant growth promotion, also, these fungi provide an increased surface area for nutrient absorption as well as to interact and accommodate many other soil organisms through their hyphae, and thus the term rhizosphere is widened to as "mycorrhizosphere" (Giri et al. 2005).

AM are considered as the transporters of various elements from rhizospheric soil to host plant. Elements which are immobile such as phosphorous, copper and zinc are of special significance as their availability towards plants is limited. Plants growing under such conditions where they face deficiency of such immobile elements in soil are greatly benefitted by this plant-fungi association (Chu 1999). AM fungi also, help plants to sustain under drought conditions by providing increased water uptake (Augé 2001), under high salt concentrations in soil (Ruiz-Lozano 2003) and heavy metal contaminated sites such as mine spoils and PHP contaminated sites (Barea et al. 2005; Boer et al. 2005).

Variety of plants which play host to AM fungi can survive in an environment where their AM partners are not present, but in natural ecosystem their association is beneficial to the plant growth promotion by significantly contributing towards nutrient uptake, increased plant biomass, offering the plant improved resistance towards pathogens and stress (Smith and Read 2008). On the other hand, AM fungi are unable to complete their life cycle without making an association with the host plant as they can only absorb the carbon assimilates from inside the plant cells; these fungi are dependent upon their plant partners for their growth and reproduction which makes them obligate biotrophs. These are asexual fungi, the phenomenon of anastomosis is observed between genetically distinct AM strains (Croll et al. 2009; Hijri and Sanders 2005). The hyphae are aseptate and coenocytic in nature, which facilitates the translocation of free flowing nuclei across the cytoplasmic streams within the hyphae (Bago et al. 1999). Due to these unique characteristics, high levels of heterozygosity and absence of a uninucleate stage in their life cycle, classical molecular approaches have failed to provide the basis of their growth and functionality, however, fast evolving tools based on transcriptomics and proteomics provided a partial approach where it is assumed that the plants are the determining factors of the symbiotic association.

12.3 Mechanism of Plant Root Colonization by Arbuscular Mycorrhizal Fungi

12.3.1 Physical

Communication between plant and fungus occurs through morphological changes in the cell structure of both host and AM (Parniske 2008). Physically diameter of mycorrhizal mycelium is relatively much smaller than the plant root or root hair,

hence can explore the soil and soil aggregates much efficiently facilitating the host in many ways (Rillig et al. 2002; Wilson et al. 2009). The process of colonization occurs in a number of morphologically well defined stages. The germinating spore physically approaches the plant root forming a structure called as appressorium, from which hyphal penetration occurs and the fungus accommodates itself by proliferating in the cortical parenchyma (Gianinazzi-Pearson 1996). In spite of accommodating inside the cortical cells of the roots the two partners in this mutualistic relationship remain separated by a zone of demarcation termed as Symbiosome, the bidirectional flow of nutrients take place at this site (Bucher et al. 2009).

12.3.2 Biochemical

The mechanism of AM interaction starts even before the mycelium is physically in contact with the host roots; in response to plant root exudates the resting spore germinates a short mycelium which repetitively extends its branches towards approaching the host roots. The AM symbiosis involves a number of signaling pathways which are partially characterized and are identical to those shared by *Rhizobium*- legume symbiosis (Kuhn et al. 2010). The attachment of pre-symbiotic mycelium to the plant cell is mediated by the host root exudates recognized as Strigolactones (Akiyama et al. 2005; Besserer et al. 2006). Diffusion of these compounds into the soil to short distances leads to the attachment of hyphopodium to the cell surface and formation of PPA (pre-penetration apparatus) in the cytoplasm of epidermal cells and outer cortical cells, the intercellular hyphae profusely branches along the axis forming branched structures called arbuscules which are the site of active nutrient exchange (Bonfante and Genre 2010).

12.3.3 Molecular

At least seven genes (SYM genes) are required for the symbiosis to establish in both cases but none of them is considered responsible for AM fungi only (Parniske 2008). Plants have membrane proteins that code for receptor like kinases (e.g. *LjSYMRK* in *Lotus japonicas; MtDMI2* in *Medicago truncatula*) which plays instrumental role in directly or indirectly recognizing rhizobial and mycorrhizal signals (Markmann et al. 2008), transduction of these signals occurs by phosphorylation of the kinase domain of an unknown substrate into the cytoplasm. All the downstream elements involved in the SYM pathway collectively transduce the signals to the nucleus. However, AM fungi are also known to produce distinct biologically active compounds which in turn can induce different pathways, as it was demonstrated through experiments on Rice (Gutjahr et al. 2008) and *Medicago* (Kuhn et al. 2010) where SYM-independent regulation of AM induced gene

expression was observed. AM releases 'Myc' factors which are similar to the Nod factors released by rhizobia during symbiosis are responsible for the host root colonization (Bucher et al. 2009). It was reported by Kosuta et al. (2003) that expression of *MtENOD11* depends upon the hyphal branching of AM, it implies that the expression of *MtENOD,* a Myc factor requires presence of strigolactones.

12.4 Mycorrhizal Role in Sustainable Nutrient Mobilization and Crop Nutrition

12.4.1 Role of Extended Hyphal Network in Nutrient Uptake

The extraradical mycelium of AM fungi has length that can extend to several centimeters in soil (Rhodes and Gerdemann 1975) and their total surface area can be of many folds greater than that of the host root, the large surface area allows the better acquisition of P and other mineral nutrients (Read and Perez-Moreno 2003). Mobilization of available nitrogen from the litter to the plants is also facilitated by AM extraradical mycelium (Hodge et al. 2001). Counting on these primary characteristics of AM symbiosis, it proves to be an even stronger candidate for low input sustainable agriculture, and through its active network of mycelium it can help in mobilizing nutrients from the crop residues itself.

12.4.2 Arbuscular Mycorrhizae and P Uptake Kinetics in Plants

The P uptake kinetics followed by these fungi is very similar to that of plant roots, but there are no kinetic parameters defined for the uptake of phosphorous by extraradical hyphae. The AM hyphae is efficient in transporting P even through a steep concentration gradient and beyond root depletion zones (Harrison and van Buuren 1995). The P-carrier system is driven by a proton-motive gradient created by the ATP-demanding transport of protons out of the fungal cells (Beever and Burns 1980). Sanders and Tinker (1973) evaluated the P flux entering *Allium cepa* roots via the hyphae, based on mean number and size of entry points per cm of the roots; the P flux represented as mol cm^{-2} s^{1} was converted into P flux per unit cross section area of hyphae, this information conveyed a value of 3.8×10^{-8} mol cm^{-2} s^{1} and indicated the measure of translocated P flux within the mycorrhizal hyphae.

The rate of P absorption per unit area of root measured under standardized conditions of temperature, concentration; pH etc. is considered as the absorption capacity, this feature plays an important and deciding role in P uptake. Cress et al. (1979) reported that mycorrhized roots were more efficient in P absorption by virtue of greater affinity for P carrier system.

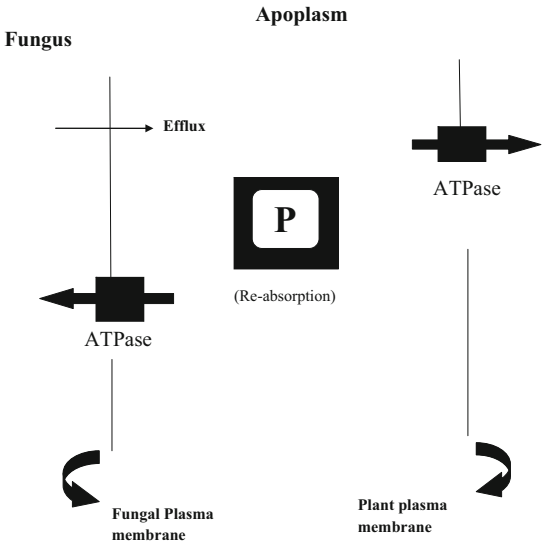


Fig. 12.1 Schematic diagram of membrane transport process involved in movement of nutrients (Smith 1993)

At the site of nutritional interchange between the host plant and fungus, active membranes are present which serves as a tripartite structure and are linked to ATP-ase activities. The intracellular space formed by AM fungi influence the necessary energy gradients required for active transport for both host and fungal partners (Fig. 12.1). AMF possess a number of P transporters such as *GiPT* found in extraradical mycelium of *Glomus intraradices* is found to have expressed in low P situation by the fungi (Maldonado-Mendoza et al. 2001). The process of P uptake through AMF can prove costly to plants some times as the fungal partners can switch their roles from being supportive to neutral (Douglas 2008; Kiers and van der Heijden 2006).

12.4.3 Arbuscular Mycorrhizae and Nitrogen Uptake

Soil consists of volumes of Amino acids that contribute towards the crop nutrition. Glutamate, Glutamine, Aspartate and Alanine are free amino acids generally found in soil, in a range of 1–10 μg g^{-1} of dry soil (Abuarghub and Read 1988). Several reports suggest that AM hyphae plays important role in utilization of inorganic N (Cruz et al. 2007; Hodge et al. 2001). Free amino acids were found in the extraradical hyphae of AM (Finlay 2008) also, maize roots colonized with mycorrhizae showed the presence of free amino acids (Cliquet and Stewart 1993) whereas, (Johansen et al. 1993) reported presence of Asparagine, Aspartate, Alanine, Glutamine and Gutamate in extraradical mycelium of *Glomus intraradices*.

Through these reports it is evident that glutamine and glutamate have a key role in incorporation of inorganic N into organic compounds and precursors for the

synthesis of rest of the amino acids in AM fungi. ^{15}N studies have shown that the N in soil is taken up by the extended extraradical hyphae as NH^{+}_{4} –N (MÄDer et al. 2000) and NO^{-}_{3} –N (Tobar et al. 1994) and translocated to plants.

12.4.4 Bacteria-AM-Legume Tripartite Symbiotic Relationship

Bacteria associated with AM also help in nutrient transport. It was observed from the example of bacteria-AM-legume tripartite symbiotic relationship, where diazotrophic bacteria fix N not only for plant but also to AM. The presence of AM fungi increases nodulation and nitrogen fixation in legumes, as AM and legume symbiosis works synergistically to provide plant growth promotion (Amora-Lazcano et al. 1998), as AM provides the plant host and rhizobacteria with P which is essential for the synthesis of enzymes required for establishing legume symbiosis and nitrogen fixation thus promotes mycorrhizal symbiosis. It is reported that rhizobium and AM in dual symbiosis can provide plant growth promotion in many legumes (Zahran 1999).

More research is still required regarding the decomposition processes in soil by mycorrhizosphere organisms as this could be significant in establishing sustainable agro ecosystem and can reduce the inputs of chemical fertilizations into fields.

12.4.5 Micronutrients Provided by Arbuscular Mycorrhizae

These fungi play an instrumental role in the translocation of zinc and copper, the content and uptake is significantly higher in the roots of mycorrhized plants in normal as well as abiotic stress condition (Porras-Soriano et al. 2009). The efficiency of extraradical hyphae to translocate copper and Zinc is high and it could contribute nearly 50–60% of the total uptake in white clover and about 25% in maize (Kothari et al. 1991; Li et al. 1991). A meta analysis of influence of AMF on Zn uptake showed the mycorrhization has positive influence on Zn concentration of plant (Lehmann et al. 2014).

High levels of Fe were reported by (Caris et al. 1998) in peanut and sorghum. It is suggested that like ectomycorrhizal fungi (Szaniszlo et al. 1981). AM also produce Fe chelating compounds, for example siderophores (Cress et al. 1986). In soil natural conditions AM compete for the Fe uptake due to presence of other soil microbes and with the root itself. The contribution towards Fe uptake therefore, is variable and dependent upon the plant-fungus combination as well as physical properties of soil. It's an advantage to AMF host plant, that they get the mineral nutrition through their fungal partner even in stressed environments (Clark and Zeto 2000).

12.5 Arbuscular Mycorrhizae Ameliorates Plant Health Through Improved Resistance to Various Abiotic and Biotic Stresses

12.5.1 Soil Salinity

Salt affected soils makes about 10% of the global land surface. Phytotoxic effects of high salinity includes lower water potential in soil solution leading to decreased water uptake, ion toxicity related to increased uptake of Na^+ and Cl^- and reduced mineral uptake and absorption. Several reports have shown the plants have better sustenance against the adverse effects of high salinity when colonized by AMF. Case studies have shown increased salt tolerance in banana plants (Yano-Melo et al. 2003) when infected by *Glomus* consortia, in cotton improved plant growth, biomass accumulation and P –nutrition varied under fungal isolate as well as severity of salt stress. Higher diversity pattern of AM fungi were found in the roots of *Lotus glaber*, a glycophytic perennial legume frequently growing in saline habitats (Mendoza and Pagani 1997; Sannazzaro et al. 2004) showed its dependence on AM colonization under P deficit conditions.

12.5.2 Soil Acidity

Soil acidification is considered to be a natural process in soil under humid environment. As the soil pH lowers to 5 and less, soluble levels of metal cation may cross threshold and become biologically toxic (Goulding 2016). Due to precipitation and adsorption, availability of P gets reduced under acidic conditions in soil. Other factors contributing soil acidification are combustion of fossil fuels, application of ammonium fertilizers, mining practices and other such human activities leads to anthropogenic soil acidification. Many reports suggest most of these soils are found in tropical agro-ecosystems, the role of AM is important as most of the crops in tropics are highly mycorrhiza-dependent in a low P status in acidic soil. The use of commercial AM inoculums can be prompted to reclaim vegetation cover having highly acidic substrate (Arines and Vilariño 1991; Corbett et al. 1996).

12.5.3 Extreme Temperature

Temperature controls the rate of all metabolic reactions; hence, temperature is a very important factor deciding the distribution of microorganism in soil around the earth. Members of Glomales are found in all kinds of temperature zone on earth and with climate their species composition is also influenced (Koske 1987). Due to slower metabolism rate at sub optimal temperature, AMF growth limits.

Extraradical mycelium of certain AM isolates can sustain freezing temperature and can reinitiate the colonization (Gavito et al. 2000, 2003). Varying temperature conditions of extreme cold to extreme high may pose adverse effect on AM and its enzymatic responses (Chen et al. 2014).

12.5.4 Water Stress

Application of AM inoculums to field is one of the research strategies used to curb the water deficiency and sustain the crop yield. During drought conditions the plant undergoes a number of changes such as physiological responses which are regulated by gene expression (Ito et al. 2006). AM symbiosis with plants has shown to improve plant productivity under water stress (Augé 2001; Ruiz-Lozano 2003; Ruiz-Sanchez et al. 2010). The extraradical hyphae of AM serves as extension to plant root and due the thin structure it can explore the soil pores and reach to the water source not available plant root not colonized by AM. Also, the hyphal assistance in water transport to plants was reported by (Khalvati et al. 2005). Some reports also support that in AM fungi the root hydraulic properties (Barzana et al. 2012; Ruiz-Lozano et al. 2009; Sanchez-Romera et al. 2016), stomatal conductance (Auge et al. 2015) and protection against oxidative damage (Ruiz-Lozano 2003). Genes putatively involved in plant response to water stress such as *P5CS*, encoding rate limiting enzyme of Pro synthesis (Porcel et al. 2004), aquaporin genes (Porcel et al. 2006), a gene (*NCED*) plays key role in ABA bio synthesis (Aroca et al. 2008) have been reported for AM colonized plants.

12.5.5 Protection Against Pathogen

A number of AMF-induced resistance was reported against the attack of microbial pathogens (Appoloni et al. 2008). Phytohormones like salicylic acid and jasmonic acid play crucial role in providing ISR (induced Systemic resistance) during pathogen invasion (Bari and Jones 2009); mycorrhization have shown to enhance the levels of these hormones significantly (Meixner et al. 2005). Besides improving uptake of nutrients, AM fungi contribute towards improved resistance to plant against soil borne pathogens. The altered resistance is manifested in physiological responses in plant metabolism through enhanced chitinolytic activity and increased rates of photosynthesis and respiration efficiency. A number of mechanism are now known such as altered exudation of plant roots contribute to change in P nutrition, production of lower molecular weight-phytoalexins, lignification of cell wall and exclusion of pathogen. Mycorrhized plants show a strong vascular system having increased flow of nutrients, higher mechanical strength and decreased effects of vascular pathogen (Huang et al. 2003). The arbuscules formed by AM fungi in plant root gets degraded during digestion of the fungus by the host that increase the

chitinolytic activity, which are effective enzymes against other fungal pathogens. Many researchers suggested production of PR proteins like chitinases (PR type 3) and β 1-3 glucanases (PR type 2) are result of this increased activity and can even work synergistically during plant-microbe interaction.

12.6 Mycorrhizal Inoculum Production and Management

12.6.1 AMF Mass Multiplication Through Trap and on Farm Production

Mass production of AM fungi is now possible using trap culture techniques using suitable host plants and different substrate such as sand, peat, expanded clay, vermiculite, per lite, soil rite, rock wool, glass beads etc. Through trap culturing the small scale production of spores is possible with in a time period of 2–3 months. This can be further used to produce large scale inocula which may involve addition of a carrier material such as charcoal or expanded clay types, where the spore can be embedded into substrate particles and can be used as direct inoculums to crops in field. Another technique of similar basis is trap culturing where the plant is uprooted from a site washed and all the traces of external soil particles are removed and then this plant can be grown in suitable sterile substrate. These types of cultures provide a mixed population which can be purified by subsequent monospore pot culturing.

On site production of inoculum can be an alternative to using commercially available inoculum.

In field experiments, it was reported on farm produced inocula performed better during field trial on tubers and yield was increased to 33–45% as compared to control treatment and commercial inoculums (Douds et al. 2005).

12.6.2 AMF Mass Multiplication Through Aeroponic and Hydroponic System

Aeroponic culturing involves growing plants under a moist environment, closed or semi-closed, where continuous spray of nutrient rich solution is facilitated for roots to profusely proliferate and giving AMF to multiply at a mass level. It is a soil less technique which provides a large number of propagule numbers even higher than the soil based inoculums. Most of all this method allows uniform and higher amount of oxygen in the rhizosphere zone. Aeroponic inoculum can be efficiently stored, transported and applied in different crops like: legume, cereal, grass, vegetable and any other herbaceous or woody plant roots. This method of inoculums production is environmentally and ecologically safe and economically viable (Coelho et al. 2014).

Another technique of AM mass multiplication is growing the fungus in a hydroponic solution. It provides the AM propagation under controlled and soil less condition. Nutrient film cultures allow the flow of large scale of fluid flowing over the plant root. MacDonald reported a system for the production of axenic culture of *Glomus caledonium* and *Trifolium parviflorum*. This system of mass multiplication faces the major challenge of limited proliferation of AM under waterlogged conditions.

12.6.3 Root Organ Cultures

The established root organ cultures were first described by White (1943). The root organ cultures have greatly improved our understanding of AM-root behavior in a compartmented system, as the propagating fungus in a dual culture system with transformed host roots can be easily studied under inverted and compound microscope. This technique can be used as a tool for AMF mass multiplication as it allows only fungus and root to grow and is free from other microbes inhabiting the rhizosphere (Fortin et al. 2002). The fungal propagules produced can be stored at 4 °C and dark for a longer period of time and can be used as inoculums for green house or field level application.

12.7 Conclusion

Enhancing crop production involves identifying factors favorable for the yield of crops and developing strategies for low input environment friendly agricultural practices. Use of biofertilizers is undoubtedly a better option over chemical fertilizers which, if not used in control amounts can, be a hazard to the soil and environment. AMF plays a key role in maintaining the soil health and its use is encouraged in agricultural practices in past many decades. Out of the major nutrients required for crops for better yield like NPK, two (N and P) can be supplemented by co inoculation of AM and rhizobacteria. Commercial inoculums is better option for field level application, higher concentration of inoculums can enhance the effects of decreased disease incidence, hence promoting yield. It is important to select the appropriate combination of plant-fungus and other organism, as this can largely influence the proliferation of extraradical hyphae, which is an important factor in plant growth promotion. An inoculum containing consortia of AM species is more robust and sustaining than inoculums with single species and helps in soil aggregation more consistently.

References

Abuarghub SM, Read DJ (1988) The biology of mycorrhiza in the Ericaceae. New Phytol 108 (4):433–441. https://doi.org/10.1111/j.1469-8137.1988.tb04184.x

Akiyama K, Matsuzaki K-I, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827. https://doi.org/10.1038/nature03608

Amora-Lazcano E, Vázquez MM, Azcón R (1998) Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. Biol Fertil Soils 27:65–70. https://doi.org/10.1007/s003740050401

Appoloni S, Lekberg Y, Tercek MT, Zabinski CA, Redecker D (2008) Molecular community analysis of arbuscular mycorrhizal fungi in roots of geothermal soils in Yellowstone National Park (USA). Microb Ecol 56:649–659

Arines J, Vilariño A (1991) Growth, micronutrient content and vesicular-arbuscular fungi infection of herbaceous plants on lignite mine spoils: a greenhouse pot experiment. Plant Soil 135:269–273

Aroca R, Del Mar Alguacil M, Vernieri P, Ruiz-Lozano JM (2008) Plant responses to drought stress and exogenous ABA application are modulated differently by mycorrhization in tomato and an ABA-deficient mutant (sitiens). Microb Ecol 56:704–719

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42. https://doi.org/10.1007/s005720100097

Auge RM, Toler HD, Saxton AM (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13–24

Bago B, Zipfel W, Williams RM, Piche Y (1999) Nuclei of symbiotic arbuscular mycorrhizal fungi as revealed by in vivo two-photon microscopy. Protoplasma 209:77–89

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69:473–488

Barzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Ruiz-Lozano JM (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

Beever RE, Burns DJW (1980) Phosphorus uptake, storage and utilization by fungi. Academic Press, London

Besserer A, Puech-Pagès V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J-C, Roux C, Bécard G, Séjalon-Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:226. https://doi.org/10.1371/journal.pbio.0040226

Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48. https://doi.org/10.1038/ncomms1046

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. https://doi.org/10.1007/s11104-008-9877-9

Bucher M, Wegmüller S, Drissner D (2009) Chasing the structures of small molecules in arbuscular mycorrhizal signaling. Curr Opin Plant Biol 12:500–507. https://doi.org/10.1016/j.pbi.2009.06.001

Caris C, Hördt W, Hawkins H-J, Römheld V, George E (1998) Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35–39. https://doi.org/10.1007/s005720050208

Chen X, Song F, Liu F, Tian C, Liu S, Xu H, Zhu X (2014) Effect of different arbuscular mycorrhizal fungi on growth and physiology of maize at ambient and low temperature Regimes. Sci World J 2014:7. https://doi.org/10.1155/2014/956141

Chu EY (1999) The effects of arbuscular mycorrhizal fungi inoculation on Euterpe oleracea mart. (açaí) seedlings. Pesquisa Agropecuária Brasileira 34:1018–1024

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902. https://doi.org/10.1080/01904160009382068

Cliquet J-B, Stewart GR (1993) Ammonia assimilation in *Zea mays* L. infected with a vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum*. Plant Physiol 101:865–871

Coelho IR, Pedone-Bonfim MVL, Silva FSB, Maia LC (2014) Optimization of the production of mycorrhizal inoculum on substrate with organic fertilizer. Braz J Microbiol 45:1173–1178

Corbett EA, Anderson RC, Rodgers CS (1996) Prairie revegetation of a strip mine in Illinois: fifteen years after establishment. Restoration Ecol 4:346–354. https://doi.org/10.1111/j.1526-100X.1996.tb00187.x

Cress WA, Throneberry GO, Lindsey DL (1979) Kinetics of phosphorus absorption by mycorrhizal and nonmycorrhizal tomato roots. Plant Physiol 64:484–487. https://doi.org/10.1104/pp.64.3.484

Cress WA, Johnson GV, Barton LL (1986) The role of endomycorrhizal fungi in iron uptake by Hilaria jamesii. J Plant Nutr 9:547–556. https://doi.org/10.1080/01904168609363465

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

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loução MA, Jakobsen I (2007) Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhizal fungi. Plant Physiol 144:782–792. https://doi.org/10.1104/pp.106.090522

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. https://doi.org/10.4141/p03-168

Douglas AE (2008) Conflict, cheats and the persistence of symbioses. New Phytol 177:849–858

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J Exp Bot 59:1115–1126. https://doi.org/10.1093/jxb/ern059

Fortin JA, Bécard G, Declerck S, Dalpé Y, St-Arnaud M, Coughlan AP, Piché Y (2002) Arbuscular mycorrhiza on root-organ cultures. Can J Bot 80:1–20. https://doi.org/10.1139/b01-139

Frank A, Trappe J (2005) On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A.B. Frank's classic paper of 1885). Mycorrhiza 15:267–275

Gavito ME, Curtis PS, Mikkelsen TN, Jakobsen I (2000) Atmospheric CO_2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (Pisum sativum L.) plants. J Exp Bot 51:1931–1938. https://doi.org/10.1093/jexbot/51.352.1931

Gavito ME, Schweiger P, Jakobsen I (2003) P uptake by arbuscular mycorrhizal hyphae: effect of soil temperature and atmospheric CO_2 enrichment. Glob Chang Biol 9:106–116

Gianinazzi-Pearson V (1996) Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell 8:1871–1883

Giri B, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmüller R, Varma A (2005) Mycorrhizosphere: strategies and functions. Soil Biol 3:213–252.

Goulding KWT (2016) Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manage 32:390–399

Graham JH (2001) What do root pathogens see in mycorrhizas? New Phytol 149:357–359

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

Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Hijri M, Sanders IR (2005) Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. Nature 433:160–163

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299

Huang J, Luo S, Zeng R (2003) Mechanisms of plant disease resistance induced by arbuscular mycorrhizal fungi. Ying Yong Sheng Tai Xue Bao 14:819–822

Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol 47:141–153

Johansen A, Jakobsen I, Jensen ES (1993) Hyphal transport by a vesicular-arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. Biol Fertil Soils 16:66–70

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biol 7:706–712

Kiers ET, van der Heijden MG (2006) Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. Ecology 87:1627–1636

Koske RE (1987) Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. Mycologia 79:55–68

Kosuta S, Chabaud M, Lougnon G, Gough C, Dénarié J, Barker DG, Bécard G (2003) A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. Plant Physiol 131:952–962

Kothari SK, Marschner H, Römheld V (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil 131:177–185

Kuhn H, Kuster H, Requena N (2010) Membrane steroid-binding protein 1 induced by a diffusible fungal signal is critical for mycorrhization in Medicago truncatula. New Phytol 185:716–733

Leal PL, Stürmer SL, Siqueira JO (2009) Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the Amazon, Brazil. Braz J Microbiol 40:111–121

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants- a meta-analysis. Soil Biol Biochem 69:123–131

Leyval C, Joner EJ, del Val C, Haselwandter K (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhäuser, Basel, pp 175–186

Li X-L, George E, Marschner H (1991) Phosphorus depletion and pH decrease at the root–soil and hyphae–soil interfaces of VA mycorrhizal white clover fertilized with ammonium. New Phytol 119:397–404

Mader P, Vierheilig H, Streitwolf-Engel R, Boller T, Frey B, Christie P, Wiemken A (2000) Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. New Phytol 146:155–161

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ (2001) A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol Plant Microbe Interact 14:1140–1148

Markmann K, Giczey G, Parniske M (2008) Functional adaptation of a plant receptor- kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6(3): e68

Meixner C, Ludwig-Müller J, Miersch O, Gresshoff P, Staehelin C, Vierheilig H (2005) Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant nts1007. Planta 222:709–715

Mendoza RE, Pagani EA (1997) Influence of phosphorus nutrition on mycorrhizal growth response and morphology of mycorrhizae in *Lotus tenuis*. J Plant Nutr 20:625–639

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Porcel R, Azcón R, Ruiz-Lozano JM (2004) Evaluation of the role of genes encoding for Δ1-pyrroline-5-carboxylate synthetase (P5CS) during drought stress in arbuscular mycorrhizal Glycine max and Lactuca sativa plants. Physiol Mol Plant Pathol 65:211–221

Porcel R, Aroca R, Azcon R, Ruiz-Lozano JM (2006) PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. Plant Mol Biol 60:389–404

Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, Azcón 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

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytol 157:475–492. https://doi.org/10.1046/j.1469-8137.2003.00704.x

Rhodes LH, Gerdemann JW (1975) Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol 75:555–561

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238:325–333

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317. https://doi.org/10.1007/s00572-003-0237-6

Ruiz-Lozano JM, del Mar Alguacil M, Barzana G, Vernieri P, Aroca R (2009) Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. Plant Mol Biol 70:565–579

Ruiz-Sanchez M, Aroca R, Munoz Y, Polon 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

Sanchez-Romera B, Ruiz-Lozano JM, Zamarreno AM, Garcia-Mina JM, Aroca R (2016) Arbuscular mycorrhizal symbiosis and methyl jasmonate avoid the inhibition of root hydraulic conductivity caused by drought. Mycorrhiza 26:111–122

Sanders FE, Tinker PB (1973) Phosphate flow into mycorrhizal roots. Pesticide Sci 4:385–395. https://doi.org/10.1002/ps.2780040316

Sannazzaro AI, Álvarez CL, Menéndez AB, Pieckenstain FL, Albertó EO, Ruiz OA (2004) Ornithine and arginine decarboxylase activities and effect of some polyamine biosynthesis inhibitors on Gigaspora rosea germinating spores. FEMS Microbiol Lett 230:115–121

Smith SE (1993) Transport at the mycorrhizal interface. Mycorrhiza 5:1–4.

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London.

Szaniszlo PJ, Powell PE, Reid CPP, Cline GR (1981) Production of hydroxamate siderophore iron chelators by ectomycorrhizal fungi. Mycologia 73:1158–1174. https://doi.org/10.2307/3759685

Tawaraya K, Hirose R, Wagatsuma T (2012) Inoculation of arbuscular mycorrhizal fungi can substantially reduce phosphate fertilizer application to *Allium fistulosum* L. and achieve marketable yield under field condition. Biol Fertil Soils 48:839–843

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Nat Acad Sci 108:20260–20264

Tobar RM, Azcón R, Barea JM (1994) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. Mycorrhiza 4:105–108. https://doi.org/10.1007/bf00203769

White PR (1943) A handbook of plant tissue culture. J. Cattle, Lancaster, PA.

Wilson GW, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461

Wright SF, Anderson RL (2000) Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. Biol Fertil Soils 31:249–253. https://doi.org/10.1007/s003740050653
Yano-Melo AM, Saggin OJ Jr, Costa Maia L (2003) Tolerance of mycorrhized banana (Musa sp. cv. Pacovan) plantlets to saline stress. Agric Ecosyst Environ 95:343–348
Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Chapter 13
Comparative Analysis of Metal Uptake Potential of Hyphal Fusion Progenies of AMF and Their Parents

Rajalakshmi Muralidharan, Prasun Ray, and Alok Adholeya

Abstract The main objective of the present study was to compare 15 arbuscular mycorrhizal fungal isolates (8 parents representing 3 *Glomus* species under the same genera Glomaceae and their 7 stable hyphal fusion progenies) for their heavy metal uptake potential so that they can be recommended for soil reclamation, environmental protection, and enhanced agricultural production. *In vitro* studies were conducted with AMF cultures grown in jars with minimal media amended with or without coal ash containing heavy metals. The experimental design was completely randomized with three replicates per treatment. Uptake of ten metals namely Al, Cd, Co, Cr, Cu, Mn, Ni, Pb, Si and Zn was analyzed by atomic absorption spectrophotometer. Study results showed that heavy metal uptake by hyphal fusion progenies and their parental isolate differs considerably among themselves and also for different metals. Hyphal fusion progeny 14 and parental isolate P46 showed high tolerance for a wide range of metals.

13.1 Introduction

Coal based thermal power stations in India use low grade coal with high ash content (40–50%). Toxic elements like As, Cr, Pb, Zn, Ni, Co, Cu, Cd and Mn have been reported to be present in bottom ash. Reviews have focused on the role of mycorrhizal fungi in the uptake of heavy metals from the polluted soils and their transfer to the plants (Leyval et al. 1997). Particularly, the enhanced uptake of phosphorus (Bolan 1991) and other less mobile nutrients such as zinc, copper, and ammonium

R. Muralidharan
Department of Biotechnology, Ministry of Science & Technology, New Delhi, India

P. Ray
Noble Research Institute, LLC, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

A. Adholeya (✉)
The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi 110003, India
e-mail: aloka@teri.res.in

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_13

is well established as the main fungal benefit to the plant (Smith and Read 1977). There is a considerable proof that mycorrhizae protect the roots against heavy metal toxicity (Gildon and Tinker 1983; Dueck et al. 1986; Jones and Huchinsson 1988). From very few investigations, it is clear that, in general, the metal-ameliorating effect strongly depends on the fungal species. Hence screening for selection of suitable isolates for reclamation of ash affected sites remains an essential criterion (Ray et al. 2005). Numerous environmental factors including climate, soil condition, and species interactions simultaneously determine symbiont function (Chagnon et al. 2013). The response of mycorrhizal fungi to toxic metals is of importance for their capability in the reclamation of polluted sites and their influence on plant growth and productivity (Blaudez et al. 2000). The decrease of metal phytotoxicity by mycorrhizal fungi has been widely demonstrated (Jones and Hutchinson 1986; Dixon and Buschena 1988; Colpaert and Van Assche 1993). Binding of metals to cell walls, sequestration of metals either by binding proteins or polypeptides, or by complexation in vacuoles could alleviate the toxic effects of free metal ions in cells by effectively lowering their intracellular concentration (Ortiz et al. 1992; Gadd 1993; Galli et al. 1994; Leyval et al. 1997). All organisms, including micro-organisms, can achieve resistance to heavy metals by "avoidance", or by "tolerance" (Joho et al. 1985; Baker 1987; Turnau et al. 1996). Medeiros et al. (1993) speculated that plant host dependence on AM fungi would increase with a corresponding increase in hyphal growth.

The toxicity of Ni generally manifested by interference with other essential metal ions and induction of oxidative stress, resulting in restricted cell division and expansion, disruption of photosynthesis, inhibition of root growth and branding, leaf chlorosis, necrosis and wilting, modification in uptake of other cations etc. (Chen et al. 2009; Nagaajyoti et al. 2010). Cadmium is one of the most toxic, non-essential and mobile metallic element found in soils (Qadir et al. 2004; Rahmanian et al. 2011) and is discharged into the soil at the rate of 22,000 tons per year globally (Anjum et al. 2008). Cadmium is a particularly dangerous pollutant due to its high toxicity and great solubility in water (Lockwood 1976). At low concentrations, Cd is not toxic to plants, but at high concentrations it is a toxic and inhibits root growth and cell division in plants such as onions (Avanzi 1950; Fiskejso 1988; Liu et al. 1992) and beans (Oehlkar 1953). The study carried out by Jiang et al. (2001) indicated that Cd+ can significantly ($P < 0.005$) inhibit the root growth of garlic only above 10^{-3} M, and has a stimulatory effect on root length at lower concentrations of 10^{-6} to 10^{-5} M ($P < 0.005$). Florijn and van Beusichem (1993) found that shoot Cd concentration was positively correlated with Cd concentration in xylem exudates of maize inbred lines, suggesting that shoot Cd concentration in the maize was determined by root characteristics including root Cd concentration and Cd loading into xylem. Binding of Cd onto cell walls and accumulation of Cd in the vacuolar compartment may be regarded as two essential metal-detoxification mechanisms (Blaudez et al. 2000).

It has been demonstrated that the hyphae of the fungal symbionts permeate the soil and uptake scarce and relatively immobile elements, especially phosphorus, and also nitrogen, potassium, copper and zinc, more effectively than the root hairs

on a non-mycorrhizal plant (Gerdemann 1968; Kleinschmidt and Gerdemann 1972; Mosse 1973). Zaefarian et al. (2013) reported that in the triple-metal-contaminated soil with Cd, Co and Pb, inoculated plants had greater Co (32.56 mg kg^{-1}) and Pb (289.50 mg kg^{-1}) concentration and *G. mosseae* enhanced the translocation of heavy metals to shoot. They have concluded that AMF are able to enhance plant bioremediation ability and tolerance to heavy metal contamination by increasing plant growth and P uptake and heavy metal sequestration by increasing P uptake, affecting heavy metal compartmentation in the plant, and altering root exudates.

Arbuscular mycorrhizal fungal effects on mycorrhizosphere mediation differ and probably depend on the kind and concentration of heavy metals in the soil. Accordingly, AMF can be efficiently used for cleaning up multi-metal-contaminated soil, indicating practical applications in phytoremediation of such soils. Rhizofiltration is a process where metals are precipitated within the rhizosphere and therefore it helps to retain the contaminants in the soil and prevent further dispersal. Since, AMF are obligate symbionts and increase the surface area of the host plant roots, they play an important role in rhizofiltration of metals in contaminated soils.

AM fungi can facilitate the survival of their host plants growing on metal-contaminated land by enhancing their nutrient acquisition, protecting them from the metal toxicity, absorbing metals, and also enhancing phytostabilization and phytoextraction (Ho-Man et al. 2013). Environmental restoration of metal/salinity polluted soils by traditional physical and chemical methods demands large investments of economic and technological resources (Susarla et al. 2002). In contrast, methods based on plants are becoming more popular as these methods are safer and cheaper (Raziuddin and Hassan 2011). Recently, novel strategies for bioremediation of heavy metal-polluted soils and waters by phytoextraction and/or phytomining with (hyper) accumulator plants have led to a surge of interest in the physiology of (hyper) accumulating plant species (Haag-Kerwer et al. 1999).

Environmental pollution by heavy metals is a major global problem and because of its toxic effects on human, animal health and environment, there is an urgent need to develop eco-friendly technologies to alleviate this problem. Metal tolerance of plants is generally increased by symbiotic, root-colonizing AMF, through metal sequestration in the AMF hyphae. The main objective of the present study was to find out the metal uptake potential of seven hyphal fusion progenies and their eight AMF parents *in vitro* so that the best metal accumulating AMF can be further utilized for phytoremediation and phytostabilization of metal contaminated soil.

13.2 Materials and Methods

In vitro study was conducted at Centre for Mycorrhizal Research Centre, The Energy and Resources Institute (TERI), New Delhi, India. Seven stable hyphal fusion progenies and their eight parental isolates of AMF were selected from The Centre for Mycorrhizal Culture Collection (CMCC), TERI, India. The eight parental AMF isolates selected for the study were *Glomus proliferum* P46, *Glomus*

proliferum P57, *Glomus proliferum* P61, *Glomus lamellosum* L7, *Glomus lamellosum* L11, *Glomus lamellosum* L36, and *Glomus lamellosum* L37 and *Glomus intraradices* I20. Seven stable hyphal fusion progenies were developed by hyphal fusion of three parental species viz., *Glomus proliferum*, *Glomus lamellosum* and *Glomus intraradices*. The seven hyphal fusion progenies selected for the study were Progeny 1 (P46xL7), Progeny 6 (P57xL7), Progeny 7 (P57xL37), Progeny 9 (P61xL36), Progeny 12 (P61xI20), Progeny 14 (L11xI20) and Progeny 16 (L36xI20). The AMF cultures [Agrobacterium mediated Ri T-DNA transformed colonized carrot roots- *Daucas carota* cv. Pusa Kesar, transformed using Ri T-DNA of *Agrobacterium rhizogenes* (strain A4, ATCC) dual culture at CMR, TERI, India] were raised on Petri-plates (Tarsons 90 mm) as well as on glass jar bottles (jam bottles 200 ml). The minimal (M) media was prepared using different chemicals and vitamins as per the standard protocol of Becard and Fortin (1988). AMF were grown on M medium as well as on M medium amended with coal ash collected from Super thermal power Station, Korba, Chattisgarh, Central India.

13.2.1 Experimental Design

After autoclaving and cooling to room temperature, jars with ash amended minimal medium were inoculated with 2 cm square blocks of stubs of inoculum containing spores and colonized roots from the edges of actively growing AMF monosporal cultures of selected AM fungi. The inoculated jars were maintained in a B.O.D incubator at 24 °C. The experimental design was completely randomized with three replicates for each treatment. Representative experiment unit with growing mycorrhizal symbiosis is shown in Fig. 13.1.

All jars were harvested after a period of 14 weeks growth by deionization of solid media using 100 ml of Sodium Citrate buffer (Doner and Becard 1991) at 37 °C in a shaker (Lab-therm-Adolf Kuhner AG, Schweiz) at 150 rpm (Biofuge Stratos, Heraeus) for 6–8 h. The culture suspension containing the spores and roots was recovered from the deionized media by wet sieving and decanting (Gerdemann and Nicolson 1963). The sieved roots with spores and hyphal biomass together were then thoroughly washed under running water to remove all traces of sodium citrate buffer as well as bottom ash. Spores and extrametrical fungal mycelium were completely separated from the roots using a brush under a stereo-zoom microscope (Leica M 10, Germany). Root samples were oven dried; dry weight was measured and ground into fine powder using a mechanical grinder. 0.2 g of powdered root samples were digested with 5 ml of HNO_3 and 1 ml of HF in a closed vessel at 170 °C (Kalra et al. 1989) using Microwave Accelerated Reaction System 5 (MARS 5), CEM Corp. USA for heavy metal analysis. The digested samples were analyzed for aluminium, nickel, cadmium, cobalt, chromium, copper, manganese, lead, silicon and zinc concentration within the colonized roots. The analysis was carried out by TJA Solutions AAS software version 1.14 (Unicam) Model SOLAAR M Series with GF 95 graphite furnace equipped with FS 95 auto sampler and the software SOLAAR M Data station version 8.12.

Fig. 13.1 AMF culture in jar

13.2.2 Statistical Analysis

The data was statistically analyzed with one-way Analysis of Variance (ANOVA—SAS Institute Inc. 1991), and differences among treatments were compared using probability of significance using Duncan's Multiple Range Test (DMRT) in Costat Statistical Software (Cohort, Berkeley, Calif) using ANOVA as $p = 0.05$ and least significant difference (LSD) values. Data was plotted to get graphical representation of results using the Microsoft Excel 2003 version. Means were separated by Least Significant Difference (LSD) at $P \leq 0.05$. After analysis of variance, results were plotted to get graphical representation of results using Microsoft Office Excel 2003.

13.3 Salient Observations

Comparative analysis of Metal uptake potential of seven AMF hyphal fusion progenies and their eight parents for selected ten metals in bottom ash amended medium is given at Table 13.1.

Table 13.1 Comparative analysis of Metal uptake potential (mg/kg) of seven AMF hyphal fusion progenies and their eight parents in bottom ash amended medium Letters represent one-way Analysis of Variance (ANOVA)

AMF	Al	Cd	Co	Cr	Cu	Mn	Ni	Pb	**Si**	**Zn**
P46	1598.78bcde	6.550g	15.3bc	96.258a	92.707de	32.554cde	39.246bc	28.473bc	5695.400a	1526.837bc
L7	1427.717cde	8.35efg	9.237cd	11.518c	91.717de	28.438cdef	32.293cd	40.033ab	1315.483fg	2017.117a
Progeny1 (P46xL7)	550.13b	6.556d	2.829c	13.053b	39.997d	11.138d	24.259e	28.473bc	1788.257def	628.282e
P57	2443.4bc	6.510g	7.373cdef	7.649c	104.937bcd	19.88efg	28.757de	39.507ab	2805.235d	1243.02bcd
Progeny6 (P57xL7)	1542.93b	13.576b	4.56bc	15.191b	77.03c	26.813b	92.537a	17.377d	1667.193ef	1036.897de
L37	597.617e	16.051b	9.627c	9.483c	93.202de	10.383e	25.787de	16.213d	658.373g	1068.85acd
Progeny7 (P57xL37)	1939.997b	7.120d	8.763b	7.034b	129.683a	17.577cd	38.277c	45.223ab	2666.113de	1322.007bcd
P61	2771.18b	10.866de	10.19c	52.551b	125.453abc	40.593bc	43.273b	48.413ab	4635.323bc	1514.7bc
L36	513.307e	7.542fg	5.957cdef	12.075c	143.497a	24.937def	24.947de	33.983bc	2173.450def	1702.389ab
Progeny9 (P61xL36)	761.795b	9.728cd	3.45c	7.672b	117.52ab	32.102b	44.1b	29.307bc	4233.930c	1710.08ab
I20	1069.867de	10.765de	5.53cdefg	24.664c	116.137bcd	33.25cd	42.163b	36.81ab	4364.657c	1171.837cd
Progeny12 (P61xI20)	793.937b	8.097d	4.347bc	81.5297a	96.64bc	52.487a	27.943d	37.857abc	5526.430ab	1369.43bcd
L11	695.067de	7.330fg	2.173g	10.653c	99.207bcde	33.25cd	33.217cd	22.403cd	2223.870def	1149.616cd
Progeny14 (L11xI20)	1529.633a	24.187a	22.33a	19.671b	106.943ab	60.359a	21.672e	48.493ab	2078.040def	1541.906bc
Progeny16 (L36xI20)	6824.42a	11.507bc	5.72bc	18.792b	114.307ab	28.677bc	31.283d	55.603a	2771.990d	1540.962bc
LSD (0.05)	1102.287	2.545	4.1601	15.896	23.6098	11.601	8.204	14.304	916.304	413.791

Values with different letters indicate significant difference at $p \leq 0.05$. Same letters within the same column indicate non-significant differences. Degree of freedom = 30

13.3.1 Uptake of Aluminium (Al)

Among the eight parental isolates, maximum Al uptake was recorded by P61 (2771.18 mg/kg) followed by P57 (2443.4 mg/kg) and P46 (1598.78 mg/kg) respectively. Least Al uptake was recorded by L36 (513.307 mg/kg). Among the seven hyphal fusion progenies, maximum Al uptake (6824.42 mg/kg) was recorded by Progeny 16, followed by Progeny 7 (1939.997 mg/kg) and Progeny 6 (1542.93 mg/kg); least uptake was observed in roots colonized with Progeny 1 (550.13 mg/kg). Highest Al uptake was observed in roots colonised with Progeny 16 followed by P61 and P57 (Fig. 13.2); when data of all 15 treatments in the ash amended medium were analysed together, least Al uptake was recorded by roots colonized with L36. Pair wise comparison between roots colonised with individual hyphal fusion progeny with their respective parents was done, roots colonised with hyphal fusion progeny 14 and 16 showed higher Al uptake than their both parents whereas roots with Progeny 6 and Progeny 7 recorded higher Al uptake than one of their parents.

13.3.2 Uptake of Cadmium (Cd)

Among the eight parental isolates, maximum Cd uptake was recorded by roots colonised with L37 (16.051 mg/kg) followed by P61 (10.867 mg/kg) and I20

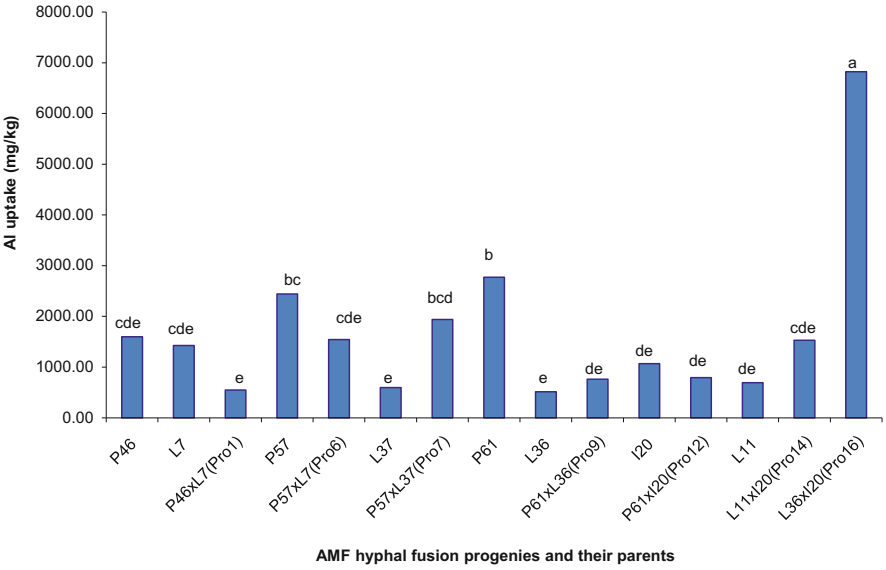


Fig. 13.2 Uptake of Al by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

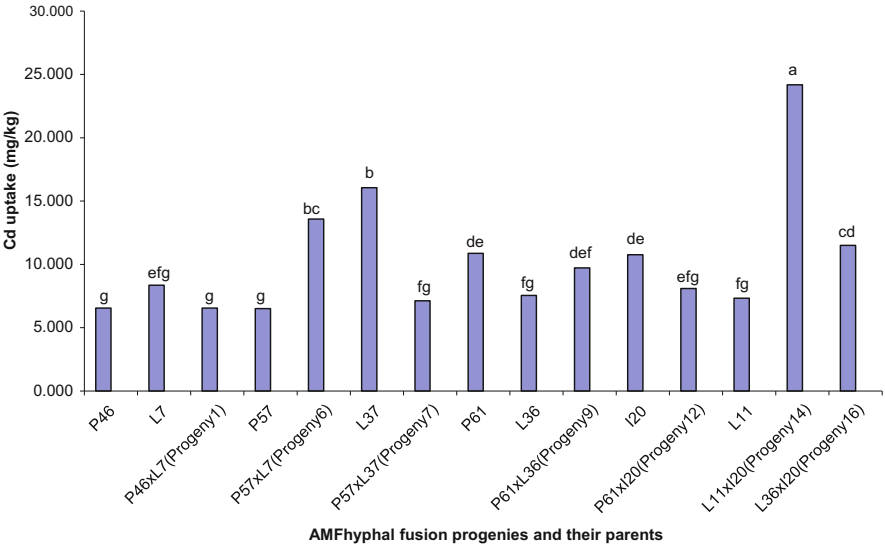


Fig. 13.3 Uptake of Cd by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

(10.767 mg/kg) and the least uptake was recorded by P57 (6.510 mg/kg). Among the seven hyphal fusion progenies, maximum Cd uptake was recorded by Progeny 14 with 24.187 mg/kg followed by Progeny 6 with 13.576 mg/kg and Progeny 16 with 11.507 mg/kg; least Cd uptake was observed in roots colonized with Progeny 1 with 6.556 mg/kg. Among all 15 AMF isolates, highest Cd uptake was observed in roots colonised with Progeny 14 followed by L37 and Progeny 6 and the least Cd uptake was recorded in roots colonized with P57 (Fig. 13.3). Though no significant level of variance was observed in the ANOVA analysis of roots colonised with hyphal fusion Progeny 1 and its parents P46 and L7, the uptake by Parent L7 was considerably higher than that of Progeny 1 and the other parent i.e. P46. Progeny 6, 14 and 16 showed higher Cd uptake than their both parents whereas roots with Progeny 1, 6, 7, and 9 recorded higher Cd uptake than one of their parents.

13.3.3 Uptake of Cobalt (Co)

Among the seven hyphal fusion progenies, maximum Co uptake was recorded by Progeny 14 with an uptake of 22.33 mg/kg followed by Progeny 7 showing an uptake of 8.77 mg/kg and Progeny 16 with 5.72 mg/kg uptake; least Co uptake (2.83 mg/kg) was observed in roots colonized with Progeny 1. Among eight parental isolates used in the study, P46 showed highest uptake (15.3 mg/kg)

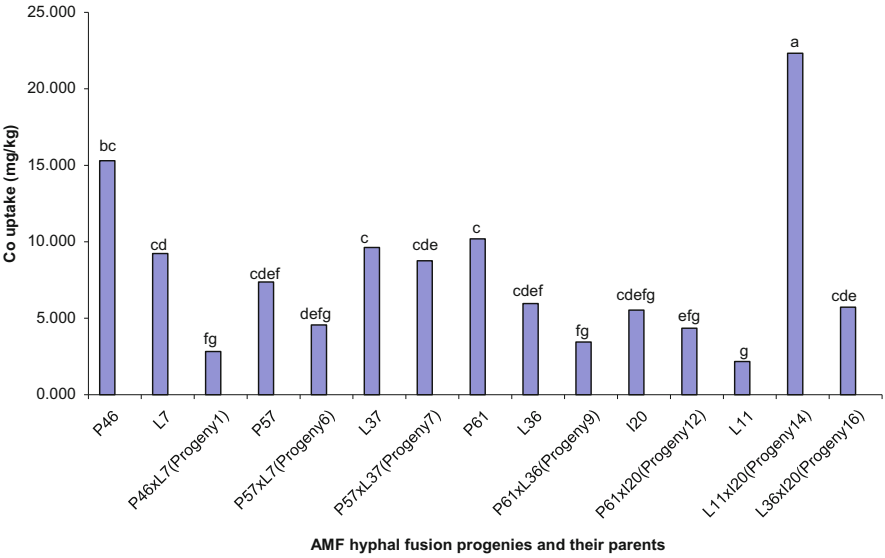


Fig. 13.4 Uptake of Co uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

followed by P61 (10.19 mg/kg) and L37 (9.63 mg/kg), and the least uptake was recorded by L11 (2.173 mg/kg). Highest Co uptake was shown by Progeny 14 followed by P46 and P61; least uptake was recorded by roots colonized with L11 (Fig. 13.4). When data of all 15 treatments was analysed together, pair wise comparison of individual progeny with their parents for Co uptake was done. Progeny 14 showed higher uptake than its both parents whereas Progeny 7 and Progeny 16 showed higher Co uptake than one of their parents.

13.3.4 Uptake of Chromium (Cr)

Among the eight parental isolates, maximum Cr uptake was recorded by P46 (96.258 mg/kg) followed by P61 (52.551 mg/kg) and I20 (24.664 mg/kg); least uptake was recorded by P57 (7.651 mg/kg). Among the seven hyphal fusion progenies, maximum Cr uptake was recorded by Progeny 12 (P61xI20) with an uptake of 81.5297 mg/kg followed by Progeny 14 (L11xI20) with 19.671 mg/kg and Progeny 16 (L36xI20) with 18.792 mg/kg; least uptake was observed in roots colonized with Progeny 7 (P57xL37) with a value of 7.034 mg/kg. Highest Cr uptake was shown by P46 followed by Progeny 12 and P61; least Cr uptake was recorded by roots colonized with Progeny 7 when data of all 15 treatments in the ash medium were analysed together (Fig. 13.5). Progeny 6 (P57xL7) and Progeny

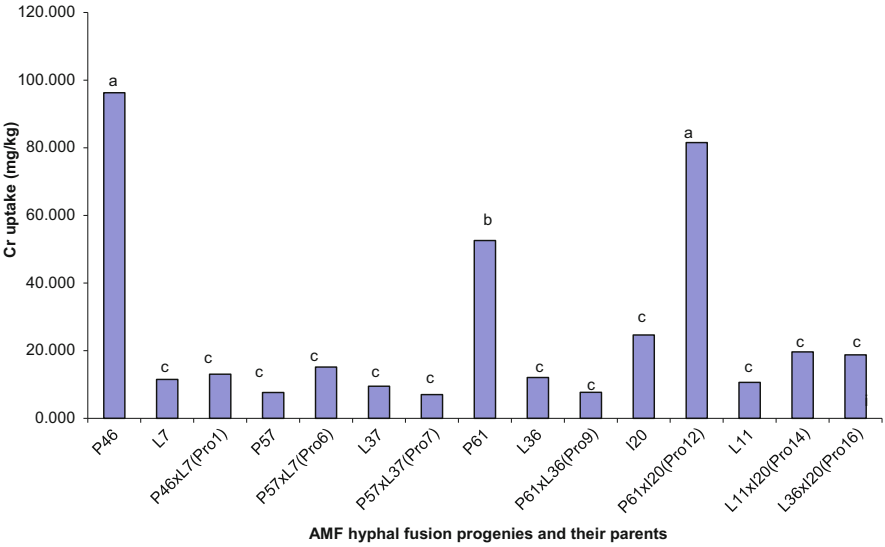


Fig. 13.5 Uptake of Cr uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

12 showed highest Cr uptake than their both parents whereas Progeny 1 (P46xL7), Progeny 14 and Progeny 16 showed higher Cr uptake than one of their parents.

13.3.5 Uptake of Copper (Cu)

Among the seven hyphal fusion progenies, maximum Cu uptake was recorded by Progeny 7 (129.683 mg/kg) followed by Progeny 9 with 117.52 mg/kg and Progeny 16 with 114.31 mg/kg; least uptake was observed in roots colonized with Progeny 1 with 39.99 mg/kg. Among eight parental isolates used in the study, L36 showed highest uptake (143.497 mg/kg) followed by P61 (125.46 mg/kg) and I20 (116.14 mg/kg), and the least uptake was recorded by L7 (91.72 mg/kg). Highest Cu uptake was observed in roots colonised with L36, followed by Progeny 7, and P61; least Cu uptake was recorded by roots colonized with Progeny 1 when data of all 15 treatments was analysed together (Fig. 13.6). Metal uptake data for individual hyphal fusion progenies were compared with their parents. Progeny 7 recorded highest Cu uptake than its both parents (P57 and L37); Progeny 14 recorded more uptake than one of its parents (L11); Progeny 1 recorded least Cu uptake than its both parents; Progenies 6, 9, 12, and 16 recorded least Cu uptake than their both parents. Statistically no significant level of variance was observed in Cu uptake when Progeny 9 and 14 were compared with their parents.

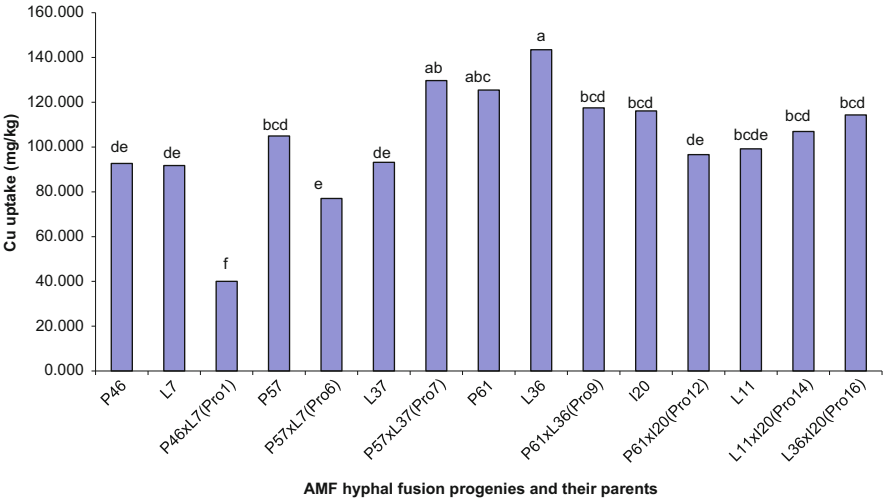


Fig. 13.6 Uptake of Cu uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

13.3.6 Uptake of Manganese (Mn)

When the Mn uptake was compared among the eight parental isolates, maximum uptake was recorded by L11 (45.89 mg/kg) followed by P61 (40.59 mg/kg) and I20 (33.25 mg/kg) respectively; least uptake was recorded by L37 (10.38 mg/kg). Among seven hyphal fusion progenies, maximum uptake was recorded by Progeny 14 (L11xI20) with an uptake of 60.359 mg/kg followed by Progeny 12 (P61xI20) with 52.49 mg/kg and Progeny 9 (P61xL36) with 32.10 mg/kg; least uptake was shown by roots colonized with Progeny 1 (P46xL7) with 11.14 mg/kg. Highest Mn uptake was recorded by Progeny 14 followed by Progeny 12 and L11 when data of all 15 treatments were analysed together, (Fig. 13.7); least uptake was recorded by roots colonized with L37 (10.39 mg/kg). Progeny 12 and Progeny 14 recorded maximum Mn uptake than their respective parents; Progeny 6, 7, 9 and 16 recorded maximum Mn uptake than one of their parents.

13.3.7 Uptake of Nickel (Ni)

Among eight parental isolates, maximum Ni uptake was recorded by L11 (33.22 mg/kg) followed by P61 (43.28 mg/kg) and I20 (42.163 mg/kg) respectively; and least uptake was recorded by L37 (25.79 mg/kg). Among the seven hyphal fusion progenies, maximum Ni uptake was recorded by Progeny 6 (P57xL7) with

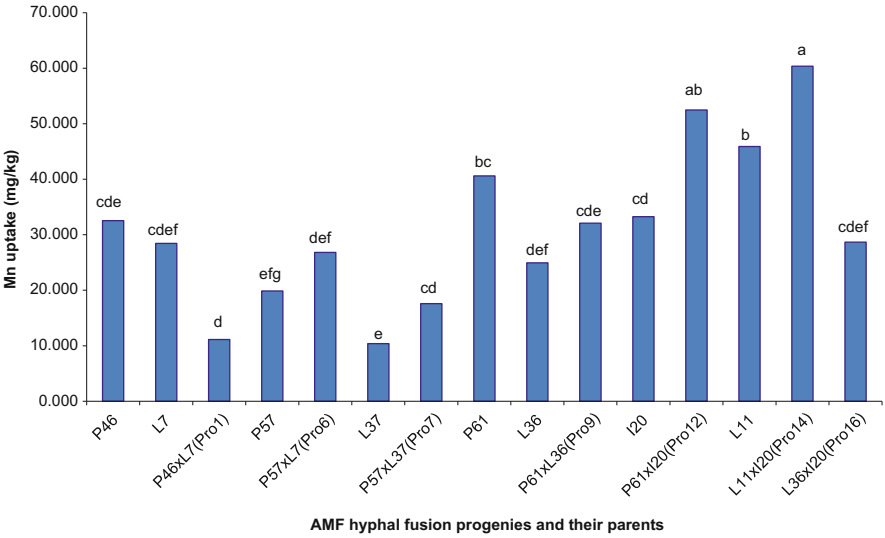


Fig. 13.7 Uptake of Mn uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

92.54 mg/kg followed by Progeny 9 (P61xL36) with 44.1 mg/kg and Progeny 7 (P57xL37) with 38.2 mg/kg; and least uptake was observed in roots colonized with Progeny 14 (L11xI20) with 21.67 mg/kg. Highest Ni uptake was recorded by Progeny 6 followed by Progeny 9 and P61; least uptake was recorded by roots colonized with Progeny 14 when data of all 15 treatments were analysed together, (Fig. 13.8). When each hyphal fusion progeny was compared with its parents, Progeny 12 and 14 recorded maximum Ni uptake than their parents; Progenies 6, 7, 9 and 16 recorded higher uptake than one of their parents; Progeny 1 recorded least Ni uptake than its parents.

13.3.8 Uptake of Lead (Pb)

Among the eight parental isolates, maximum Pb uptake was recorded by P61 (48.41 mg/kg) followed by L7 (40.03 mg/kg) and P57 (39.507 mg/kg) respectively; and least uptake was recorded by L37 (16.21 mg/kg). Among the seven hyphal fusion progenies, maximum Pb uptake was recorded by Progeny 16 (L36xI20) with an uptake of 55.60 mg/kg followed by Progeny 14 (L11xI20) with 48.493 mg/kg and Progeny 7 (P57xL37) with 45.22 mg/kg; least Pb uptake was observed in roots colonized with Progeny 6 (P57xL7) with 17.377 mg/kg. When data of all 15 treatments in the ash medium were analysed together (Fig. 13.9), highest Pb uptake was observed in roots colonised with Progeny 16 (55.60 mg/kg) followed by Progeny

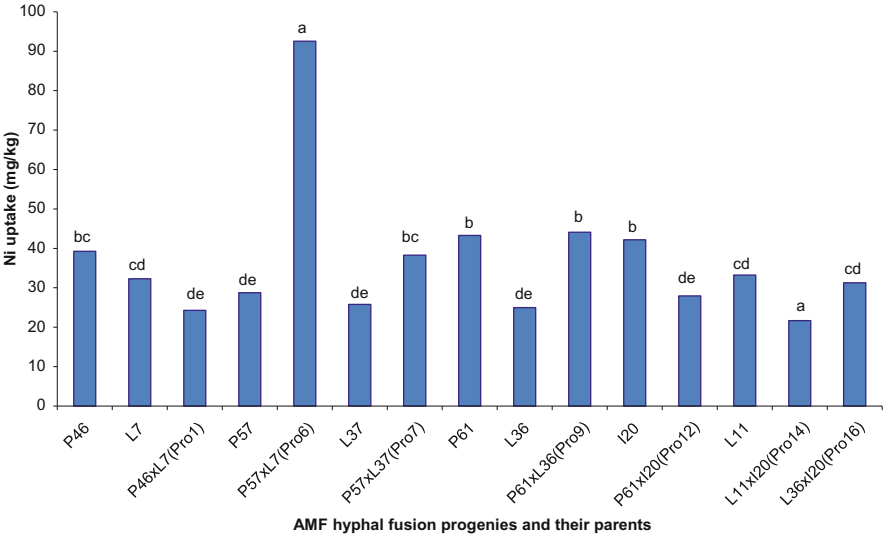


Fig. 13.8 Uptake of Ni uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

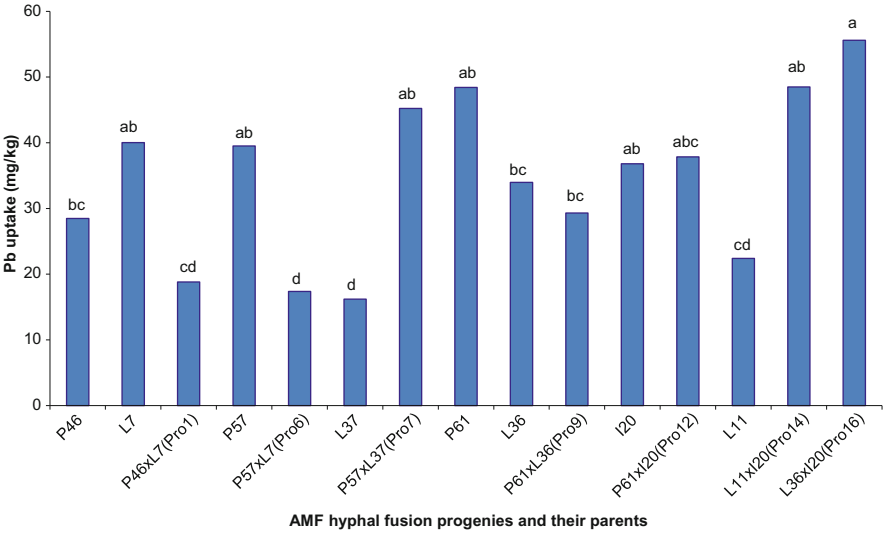


Fig. 13.9 Uptake of Pb uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

14 (48.493 mg/kg) and P61 (48.413 mg/kg); and least Pb uptake was recorded by roots colonized with L37 (16.21 mg/kg). When each hyphal fusion progeny was compared with its parents, Progenies 7, 14 and 16 recorded maximum uptake than

their respective parents; Progeny 12 recorded higher uptake than one of its parents; Progenies 1, 6 and 9 recorded least Pb uptake than their respective parents.

13.3.9 Uptake of Silicon (Si)

Among the seven hyphal fusion progenies, maximum Si uptake was recorded by Progeny 12 (5526.43 mg/kg) followed by Progeny 9 (4233.93 mg/kg) and Progeny 16 (2771.99 mg/kg) respectively; and least uptake was recorded by Progeny 6 (1667.193 mg/Kg). Among the eight parental isolates of AMF, maximum Si uptake was recorded by P46 with an uptake of 5695.4 mg/kg followed by P61 with 4635.323 mg/kg and I20 with 4364.66 mg/kg uptake; least uptake was observed in roots colonized with L37 (658.37 mg/kg). When data of all 15 treatments were analysed together (Fig. 13.10), highest uptake was recorded by P46 followed by Progeny 12 and P61; and least uptake was recorded by roots colonized with L37. When each hyphal fusion progeny was compared with its parents for Si uptake, Progeny 7, 14 and 16 recorded maximum uptake than their respective parents; Progenies 1 and 6 recorded less uptake than their both parents; and Progeny 12 recorded more Si uptake than one of its parents.

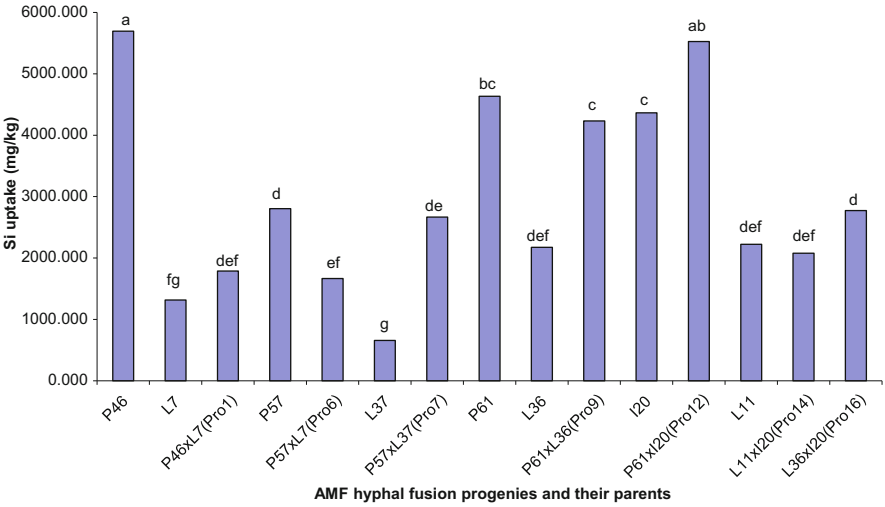


Fig. 13.10 Uptake of Si uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

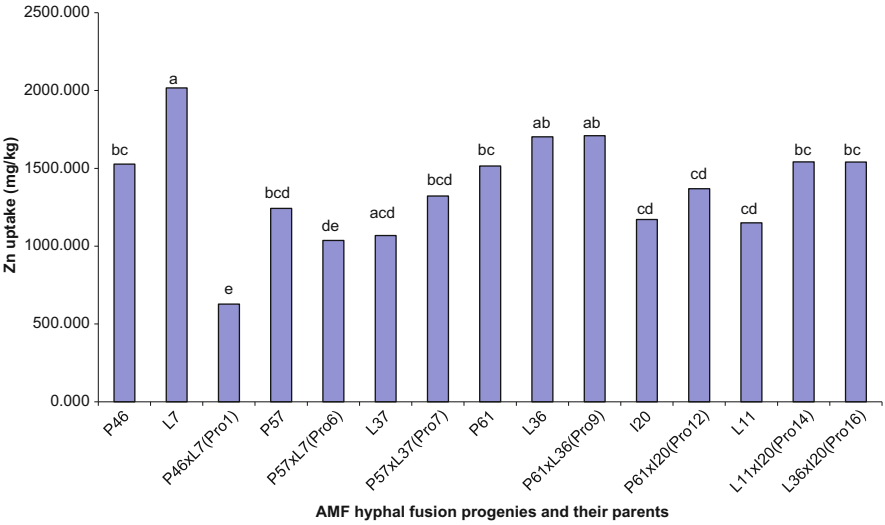


Fig. 13.11 Uptake of Zn uptake by roots colonised with 7 AMF hyphal fusion progenies and their parents in bottom ash amended medium. Letters represent Analysis of Variance (ANOVA) with significant difference at $P \leq 0.05$

13.3.10 Uptake of Zinc (Zn)

Comparison of Zn uptake among the eight parental isolates showed that maximum uptake was recorded by L7 (2017.27 mg/kg) followed by L36 (1702.389 mg/kg) and P46 (1526.84 mg/kg); lowest uptake was recorded by L37 (1068.85 mg/kg). Among the seven hyphal fusion progenies, maximum uptake was recorded by Progeny 9 with an uptake of 1710.08 mg/kg followed by Progeny 14 with 1541.91 mg/kg and Progeny 16 with 1540.97 mg/kg; least uptake was observed in roots colonized with Progeny 1 with 628.28 mg/kg. When data of all 15 treatments were analysed together, highest uptake was recorded by L7 (2017.12 mg/kg), followed by Progeny 9 (1710.08 mg/kg) and L36 (1702.389 mg/kg); least uptake was recorded by roots colonized with Progeny 1 (Fig. 13.11). When each hyphal fusion progeny was compared with its parents, it was observed that Progenies 14 and 16 recorded maximum uptake than their parents; and Progenies 1, 6 and 7 recorded maximum uptake than one of their parents; Progenies 9 and 12 recorded less Zn uptake than their respective parents.

13.4 Interpretation

AMF are commonly occurring soil microbes that have a symbiotic association with their host plant and hence significantly affect its activities and growth (Klironomos 2003). The function of AMF depends on the efficiency with which the fungal

symbiont absorbs inorganic and/or organic available nutrients from soil (Marschner and Dell 1994). Zaefarian et al. (2013) have reported that kind and concentration of contamination affected the response of *G. mosseae* to added heavy metals. In the control treatments where plants absorbed more Pb, Cd, and Co than inoculated ones, AMF might have selectively contributed to the exclusion of toxic and nontoxic elements (i.e., mycorrhizoremediation). Metals may be sequestered in the hyphae and not translocated to the plants and these results are in agreement with reports of Zhou (1999). Leyval et al. (1997) reported that inconsistent results on the effects of AMF on heavy metal uptake may be a consequence of a wide range of factors such as the inherent heavy metal uptake capacity of plants, plant root density, corresponding fungal properties and soil adsorption/desorption characteristics. Results presented in this study agree with these reports, because selected hyphal fusion progenies and their parents showed varying effect on metal uptake. In the present study, Progeny 16 showed highest Al and Pb uptake potential; Progeny 14 showed maximum effect for Cd, Co and Mn uptake; parental isolate P46 had highest effect for Cr and Si uptake; parental isolate L36 best for Cu uptake; Progeny 6 for Ni uptake; and parental isolate L7 best for Zinc uptake. Among the seven hyphal fusion progenies, highest effect was shown by Progeny 16 for Al and Pb uptake; Progeny 14 for Co, Cd and Mn uptake; Progeny 6 for Ni uptake; Progeny 12 for Cr, Mn and Si uptake; Progeny 7 for Cu uptake; and Progeny 9 for Zn and Ni uptake. When metal uptake efficiency among the eight parental isolates was considered, P61 was best for Al and Ni; L37 for Cd; P46 for Co, Cr, Fe and Si; L36 for Cu; and L11 for Mn. On the basis of the metal uptake data, Progeny 14 and parental isolate P46 can be considered as good candidates for various metal uptake, in the current study.

These results are significant in view of the report of Zaefarian et al. (2013) that heavy metal uptake by plants from artificially contaminated soils often differs from the uptake in geogenic contaminated soils with the same level of contamination. Chaudhry et al. (1998, 1999) have also reported that mycorrhizae have been found in plants growing on naturally heavy metal–contaminated lands. Khan (2005) has reported that it is important to use indigenous AMF strains, which are best adapted to actual soil and climatic conditions, to produce site-specific AMF inocula. Hence these study results are to be tested in actual contaminated sites for verification and confirmation. As reported by Gaur and Adholeya (2004) there is need to develop new methods and optimize the conditions to grow in large quantities and characterize, develop and screen large number of AM fungi for tolerance to metals.

Our results revealed that a single hyphal fusion progeny or parental isolate cannot be identified as the best AMF for uptake of a variety of metals but its potential vary for each metal and is also dependent on the medium in which it is grown. This is in agreement with Weissenhorn et al. (1995) who reported that influence of AM on plant metal uptake depends on plant growth conditions, on the fungal partner and on the metal, and cannot be generalized. Therefore, it is concluded that Progeny 14 and P46 showed high tolerance for a wide range of metals and are best suitable for heavy metal uptake in polluted sites and reclamation

areas. It is important to use these results for application purposes in metal polluted soils. Further research is required to be undertaken to substantiate these findings.

Acknowledgements This work was carried out as a part of the Ph.D thesis of the first author at TERI University, New Delhi. We wish to acknowledge the technical support and guidance provided by TERI University, New Delhi. We thank TERI, New Delhi for providing the infrastructural facilities. We also acknowledge with thanks the financial support obtained from Department of Biotechnology, New Delhi. Thanks are also acknowledged to the technical support provided by Dr. Kamlesh Kumar Shukla, Mr. U.G. Reddy and Mr. H.S. Uppal of TERI, New Delhi.

References

Anjum NA, Umar S, Ahmad A, Iqbal M, Khan NA (2008) Ontogenic variation in response of *Brassica campestris* L. to cadmium toxicity. J Plant Interact 3:189–198

Avanzi M (1950) Observationi sull'attivita cytological di alcuni compost chimic. Caryologia 3:234–248

Baker AJM (1987) Metal tolerance. New Phytol 106:93–111

Becard G, Fortin JA (1988) Early events of vesicular arbuscular mycorrhizal formation on Ri-T-DNA transformed roots. New Phytol 108:211–218

Blaudez D, Botton B, Chalot M (2000) Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiol 146:1109–1117

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil 134:189–207

Chagnon PL, Bradley RL, Maherali H, Klironomos JN (2013) A trait-based framework to understand life history of mycorrhizal fungi. Trends Plant Sci 18:484–491

Chaudhry TM, Hayes WJ, Khan AG, Khoo CS (1998) Phytoremediation focusing on accumulator plants that remediate metal contaminated soils. Aust J Ecotoxicol 4:37–51

Chaudhry TM, Hill L, Khan AG, Kuek C (1999) Chapter 27: Colonization of iron and zinc-contaminated dumped filter cake waste by microbes, plants, and associated mycorrhizae. In: Wong MH, Wong JWC, Baker AJM (eds) Remediation and management of degraded land. CRC Press, Baca Roton, FL, pp 275–283

Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: recent advances and future prospects. CLEAN Soil Air Water 37:304–313

Colpaert JV, Van Assche JA (1993) The effects of cadmium on ectomycorrhizal *Pinus sylvestri*s L. New Phytol 123:325–333

Dixon RK, Buschena CA (1988) Response of ectomycorrhizal *Pinus banksiana* and *Picea glauca* to heavy metals in soil. Plant Soil 105:265–271

Doner LW, Becard G (1991) Solubilization of gellan gels by chelation of cations. Biotechnol Tech 5:25–28

Dueck TA, Visser P, Ernst WHO, Schat H (1986) Vesicular-arbuscular mycorrhiza decrease zinc toxicity to grasses in zinc polluted soil. Soil Biol Biochem 18:331–333

Fiskejso G (1988) The Allium test- an alternative in environmental studies: the relative toxicity of metal ions. Mutat Res 197:243–260

Florijn PJ, van Beusichem ML (1993) Cadmium distribution in maize inbred lines: effects of pH and level of Cd supply. Plant Soil 153:79–84

Gadd GM (1993) Interactions of fungi with toxic metals. Tansley Review No 47. New Phytol 124:25–60

Galli U, Schuepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plant 92:364–368

Gaur A, Adholeya A (2004) Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Curr Sci 86:528–534
Gerdemann JW (1968) Vesicular-arbuscular mycorrhiza and plant growth. Annu Rev Phytopath 6:397–418
Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244
Gildon A, Tinker PB (1983) Interactions of vesicular arbuscular mycorrhizal infection and heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol 95:247–261
Haag-Kerwer A, Schafer HJ, Heiss S, Walter C, Rausch T (1999) Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. J Exp Bot 50:1827–1835
Ho-Man L, Zhen-Wen W, Zhi-Hong Y, KinLam Y, Xiao-Ling P, Kwai-Chung C (2013) Interactions between arbuscular mycorrhizae and plants in phytoremediation of metal-contaminated soils: a review. Pedosphere 23:549–563
Jiang W, Liu D, Hou W (2001) Hyperaccumulation of cadmium by roots, bulbs and shoots of garlic (*Allim sativum* L.) Bioresour Technol 76:9–13
Joho M, Imai M, Murayamma T (1985) Different distribution of Cd^{2+} between Cd-sensitive and Cd-resistant srains of *Saccharomyces cerevisiae*. J Gen Microbiol 131:53–56
Jones MD, Hutchinson TC (1986) The effect of mycorrhizal infection on the response of *Betulapa pyrifera* to Nickel and Copper. New Phytol 102:429–442
Jones MD, Hutchinson TC (1988) Nickel toxicity in mycorrhizal birch seedlings infected with *Lactarius rufus* or *Scleroderma flavidum* I. Effects on growth, photosynthesis, respiration and transpiration. New Phytol 108:451–459
Kalra YP, Marynard DG, Radford FG (1989) Microwave digestion of tree foliage for multi-element analysis. Can J For Res 19:981–985
Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364
Kleinschmidt GD, Gerdemann JW (1972) Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. Phvtopathology 62:1447
Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301
Leyval C, Haselwandter K, Tarnau K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological ecological and applied aspects. Mycorrhiza 7:139–153
Liu DH, Jiang WS, Li MX (1992) Effects of Cd2+ on root growth and cell division of *Allium cepa*. Acta Sci Circumstantiae 12:439–446
Lockwood MP (1976) Effects of pollutants on aquatic organisms. Cambridge University Press, New York
Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102
Medeiros CAB, Clark RB, Ellis JR (1993) Effects of MES [2(N-Morpholino)-ethane sulfonic acid] and pH on mineral nutrient uptake by mycorrhizal and nonmycorrhizal maize. J Plant Nutr 16:2255–2272
Mosse B (1973) Growth responses to vesicular-arbuscular mycorrhiza IV. In: Soil given additional phosphate. New Phytol 72:127–136
Nagaajyoti PC, Lee KD, Sreekanth TVM (2010) Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett 8:199–216
Oehlker J (1953) Chromosome breaks influenced by chemicals. Heredity 6:95–105
Ortiz DF, Kreppel L, Speiser DM (1992) Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter. EMBO J 11:3491–3499
Qadir S, Qureshi MI, Javed S (2004) Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. Plant Sci 167:1171–1181

Rahmanian M, Habib K, Younes RD, Mirhasan RS (2011) Effects of heavy metal resistant soil microbes inoculation and soil Cd concentration on growth and metal uptake of millet, couch grass and alfalfa. Afr J Microbiol Res 5:403–410

Ray P, Reddy UG, Lapyrie F et al (2005) Effect of coal ash on growth and metal uptake by some selected ectomycorrhizal fungi in vitro. Int J Phytoremediat 7:1–18

Raziuddin F, Hassan G (2011) Effects of cadmium and salinity on growth and photosynthesis parameters of Brassica species. Pak J Bot 43:333–340

SAS Institute Inc. (1991) Proceedings of the Sixteenth Annual SAS" Users Group International Conference. Cary, NC: 1745 pp.

Smith SE, Read DJ (1977) Mycorrhizal symbiosis, 2nd edn. Academic Press, London

Susarla S, Medina VF, McCutcheon SC (2002) Phytoremediation: an ecological solution to organic chemical contamination. Ecol Eng 18:647–658

Turnau K, Miszalski Z, Trouvelot A et al (1996) *Oxalis acetosella* as a monitoring plant on highly polluted soils. In: Proceedings of the mycorrhizal conference in Granada, pp 483–486

Weissenhorn I, Leyvasl 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

Zaefarian F, Rezvani M, Ardakani MR, Rejali F, Miransari M (2013) Impact of mycorrhizae formation on the phosphorus and heavy-metal uptake of Alfalfa. Commun Soil Sci Plant Anal 44:1340–1352. https://doi.org/10.1080/00103624.2012.756505

Zhou JL (1999) Zinc biosorption by *Rhizopus arrhizus* and other fungi. Appl Microbiol Biotechnol 51:686–693

Chapter 14
Role of Mycorrhiza in Phytoremediation Processes: A Review

Manab Das, Vijay Sandeep Jakkula, and Alok Adholeya

Abstract Mycorrhizal fungi has been explored for several purposes including enhancement of crop production in marginal soil, improvement of soil health and also for providing protection to crops under environmental stress conditions. Through this chapter an attempt is made to highlight some aspects on worldwide uses of mycorrhiza for phytoremediation processes, case studies have been presented highlighting TERI's phytoremediation work across various sectors in India and abroad.

14.1 Introduction

Biosphere pollution with organics and heavy metal is accelerated dramatically during last few decades due to industrial activities such as mining, smelting, manufacturing, amendments of agricultural soils with agro chemicals etc. While our demand for new chemicals and petroleum derivatives has produced a broad spectrum of hydrocarbon contaminants, mining and associated activities have produced inorganic contaminants with rapid and wide translocation potential in the environment. On the other hand, with the increasing use of agrochemicals to maintain and improve soil fertility, unwanted elements (e.g. Cd) enter into soils due to contaminated sources of fertilizers. Accumulation of metals and metalloids in our agricultural soil pose threat to food safety issues and potential health risk due to soil to plant transfer of metal. Contaminants can also cause a detrimental effect on soil ecosystem. Thus, the needs of the hours are not just to stabilize soil and vegetation in disturbed areas but to reconvert them into productive agricultural and forest land compatible with pre-disturbed terrain, providing an integrated range of end uses. In this increasing demand for sustainable, long term approaches for the rehabilitation of disturbed soil, physico-chemical and biological remedial technologies in general have been the subject of considerable research over the last three decades. Biochemical processes such as bioleaching, bio sorption, bio oxidation or

M. Das • V.S. Jakkula • A. Adholeya (✉)
The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi 110 003, India
e-mail: aloka@teri.res.in

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_14

reduction, bio methylation of metal contaminants have shown some promises and could be used for soil sediment systems. Several other technologies including chemically enhanced soil flushing by extracting solution such as organic and inorganic acids, and complexation agents have also been proposed for remediation. But all these methods in many cases are expensive, labor intensive, and resulted in significant changes to the physical, chemical, and biological characteristics of the treated soil. High treatment cost and extensive labor requirement of such technologies have prompted to develop alternative and cheaper technologies to recover the contaminated soil or waste material. Current research in this area now includes plants to remediate polluted soils and to facilitate improvement of soil structure, the innovative techniques being known as phytoremediation. However, often physicochemical properties of waste or contaminated soil prevents plant establishment due to absence of adequate nutrients and microbial activities. In such cases, use of plants as phytosymbiont and associated mycorrhizal fungi as mycosymbiont is appeared as an alternative strategy for safe and efficient management of contaminated soil or solid waste. The mycorrhizal symbiosis has strongly co-evolved over time such that each partner depends on the other for survival and fitness in existing ecosystem. The fungi act as extensions of the root system and improve host nutrition by their ability to take up nutrients such as nitrogen, phosphorus and water more efficiently than roots alone. More interestingly, mycorrhizal fungi protect the plant from certain abiotic and biotic stresses, and produce phytohormones while fungi get benefits from plant photosynthates. Such symbiosis extends beyond the hosts as hyphal webs in the rhizosphere and can promote the transfer of nutrients to neighboring plants. To highlight the use of mycorrhizae in phytotechnologies and their potential outcomes, present article present a brief overview of some phytoremediation studies involving mycorrhiza.

14.2 Mycorrhizal Fungi and Environmental Stress

The arbuscular mycorrhizal (AM) symbiosis, an ancient interaction between plant roots and zygomycetous fungi (Morton and Benny 1990), is recognized to benefit plants under environmental stress conditions (Audet and Charest 2006; Charest et al. 1997; Subramanian and Charest 1998). They expand the interface between plants and soil environment through extraradical fungal mycelium (ERM) radiating from the colonized root cortex far into the surrounding soil and contribute to plant uptake of macronutrients such as phosphorus, nitrogen as well as micronutrients copper, and zinc. Mycorrhiza not only provide the plants with water and mineral compounds and help to improve the structure of soil, but have also been shown to act as filters, blocking xenobiotics within their mycelium resulting into reduced toxicity to the plants. They influence the physiology of their host plants making them less vulnerable to pathogens, soil pollution, salinity, drought and a number of other environmental stress factors. Furthermore, AMF also directly help the plant to escape from the build-up of phytotoxic concentrations of certain pollutants by

secreting specific detoxifying compounds (organic acids). Such plant-AMF mediated remediation mechanism is also supported by secreting plant exudates, e.g. short chain organic acids, phenolics, and small concentrations of high molecular weight compounds (enzymes and proteins) to stimulate bacterial transformations (enzyme induction); by building up of organic carbon to increase microbial mineralization rates (substrate enhancement) or by providing habitat for increased microbial populations and activity (Korade and Fulekar 2009).

However, the specific role of AMF in the host exposure to environmental stress and in the progression of the host stress response depends on a variety of factors, including the plant species and ecotype; the fungal species and ecotype; contaminants and its availability; soil edhaphic conditions, including soil fertility; and plant growth conditions, such as light intensity or root density (Pawlowska and Charvat 2004). Recent studies on application of mycorrhizal fungi in contaminated environments are presented in Table 14.1. Among the rhizosphere microorganisms involved in plant interactions with the soil, the Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) have gained prominence all over the world to treat different kind of soils (Ma et al. 2011). Mycorrhizal fungi have greatest impact on elements with narrow diffusion zones around plant roots, including heavy metals and phosphorus. An important arbuscular mycorrhizal genus is Glomus, which colonize a variety of host species, including crops and tree species. Mycorrhizal fungi are a direct link between soil and roots, and consequently of great importance in phytoremediation.

14.3 Mycorrhiza and Organic Pollutants

Mycorrhiza contributes to phytoremediation of organic contaminants loaded sites through biodegradation of respective contaminants. AMF have been reported for dissipation of organic contaminants such as atrazine (Huang et al. 2007), polycyclic aromatic hydrocarbons (PAHs) (Joner et al. 2001; Joner and Leyval 2003; Xu et al. 2006; Wu et al. 2008a), DDT (Wu et al. 2008b) and weathered p,p-DDE in soils (White et al. 2006). ECM (Ectomycorrhiza) have also been reported to degrade polychlorinated biphenyls (PCBs) and for depression in PAH dissipation (Joner et al. 2006; Genney et al. 2004). In their study, Joner and Leyval (2003) found higher dissipation rates in soil contaminated with a PAH level of 2000 mg/kg when ryegrass and white clove were colonized by AMF. Similarly, Liu et al. (2004) observed that presence of AM increased the degradation rates of benzo [a] pyrene in soils planted with alfalfa. However, PAH concentration of 100 mg/kg was found to have negative effects on degradation rate.

Till date, exact mechanisms involved in interactions between AMF and organic contaminants in soil remain unclear. It is reasonable to expect that soil microbial activity enhanced and soil microbial communities modified by AMF play a key role in the degradation of organic contaminants. In a study by Joner and Leyval (2003), PAH degradation followed a radial pattern positively correlated to root proximity,

Table 14.1 Recent studies focusing on mycorrhizae in contaminated environment

Plant species	Mycorrhizal inoculum	Contamination type	References
Pisum sativam (Pea)	*Glomus intradices*	Alkaline clay loam laden with Cd (100 mg/kg)	Rivera-Becerril et al. (2002)
Lolium perenne (Perennial ryegrass)	*Glomus mosseae*	PAH-contaminated industrial soils (400–2000 mg/kg)	Joner and Leyval (2003)
Trifolium repens (White clover)	*Glomus mosseae*	PAH-contaminated industrial soils (400–2000 mg/kg)	Joner and Leyval (2003)
	Glomus mosseae	Innert soil less substrate spiked with Cd	Vivas et al. (2003)
Zea mays (Maize)	*Glomus caledonium*	Calcareous sandy soil and loam soil, spiked with Zn (0–600 mg/kg)	Chen et al. (2004)
		Quartz sand spiked with Cu and Cd	Liao et al. (2003)
	Glomus intraradices	Sand spiked with Pb	Malcova et al. (2003)
	Glomus etunicatum	Soil spiked with atrazine	Huang et al. (2009)
Medicago sativa (Alfalfa)	*Glomus caledonium*	Soil spiked with benzo[a]pyrene (0–100 mg/kg)	Liu et al. (2004)
Alnus glutinosa (Common alder)	*Glomus intraradices*	Soil from an acetylene and polyvinylchloride factory	Oliveria et al. (2005)
Pinus sylvestris (Scots pine)	*Paxillus involutus* (ECM)	Growth substrate spiked with Cd (0–100 mg/kg)	Kim et al. (2004)
Leymus cinereus (Basin wild rye)	Site specific AM	Silica sand spiked with arsenic	Knudson et al. (2003)
Eucalyptus rostrata (Eucalyptus)	*G. deserticola*	Sandy soil spiked with Pb	Bafeel (2008)
Triticum aestivum (Wheat) and *Vigna radiate (Mungbean)*	*Glomus mosseae*	Agricultural soil spiked with Polycyclic aromatic hydrocarbons (500 mg/kg)	Rabie (2004)

supporting the hypothesis of rhizosphere microflora stimulation. Once arbuscular mycorrhizal association has developed, AMF hyphae influence the surrounding soil that has been termed the mycorrhizosphere (Linderman 1988; Giri et al. 2005), resulting in the development of distinct microbial communities in the rhizosphere and bulk soil (Andrade et al. 1997; Cheng and Baumgartner 2006; Purin and Rillig 2008). Phospholipid fatty acid (PLFA) analysis has revealed an important qualitative difference in microbial community structure in mycorrhizosphere soil as affected by AMF in PAH-spiked soil (Joner et al. 2001). Furthermore, the AM fungal hyphosphere, the zone of soil affected by the extraradical in hyphae (Marschner 1995), may support a distinct microbial community within the

mycorrhizosphere and exert effects on degradation of organic compounds in soil through enzymatic activities.

Based on various observations, researchers have examined five plant enzyme systems in sediments and soil namely dehalogenase, nitroreductase, peroxidase, laccase and nitrilase. Such release of exudates and enzymes that stimulate microbial activity, biochemical transformations and enhancement of mineralization in the rhizosphere (the root-soil interface) has been attributed to AMF and the microbial consortia (Schnoor 1997). AMF can also increase the activities of soil enzymes such as phosphatase and dehydrogenase (Dodd et al. 1987; Kothari et al. 1990; Vazquez et al. 2000). Dehydrogenase, a soil oxidoreductase, is an intracellular enzyme catalyzing oxidoreduction reactions of organic compounds. Several studies have demonstrated that the dehydrogenase enzyme activity of microorganisms is one of the most sensitive parameters available for toxicity evaluation and alkaline phosphatase is involved in the process of phosphate acquisition in mycorrhizal plants (Gianinazzi et al. 1992). For example, degradation of Atrazine, which is one of the agricultural herbicides most frequently detected in soils and waters, is associated with higher dehydrogenase (Seibert et al. 1991; Singh et al. 2004) and phosphatase activities (Perucei et al. 1988; Bielinska and Pranagal 2007, Kazemi et al. 2008). In another study, the role of mycorrhiza during the remediation of PAH in mixed sward of clover and ryegrass was examined and found enhanced losses of chrysene and dibenz(a,h)anthracene in planted soil containing a mycorrhizal inoculum (Joner et al. 2001). Different mechanisms have been proposed to explain the effect of the plant rhizosphere on PAH dissipation such as an increase in microbial numbers, an improvement in the physical and chemical soil conditions, increased humification and adsorption of pollutant in rhizosphere. However, the relative contribution of each process has not been clearly elucidated.

14.4 Mycorrhiza and Heavy Metals, Radionuclide

Heavy metals are the main group of inorganic contaminants and a considerable large area of land is contaminated with them due to mining, industry, agriculture and defense activities. Although metals are present naturally in the earth's crust at various levels and many metals are essential for cells (e.g. copper, iron, manganese, nickel, zinc), all metals are toxic at higher concentrations. Specifically, any metal (or metalloid) species may be considered a "contaminant" if it occurs where it is unwanted, or in a form or concentration that causes a detrimental human or environmental effect (McIntyre 2003).

Metal concentrations in soil typically range from less than one to as high as 70,000 mg/kg. Irrespective of the origin of the metals in the soil, excessive levels of many metals can result in soil quality degradation, crop yield reduction, and poor quality of agricultural products (Long et al. 2002). Since heavy metals are not biodegradable and may enter the food chain, they are a long-term threat to both the environment and human health (Jarup 2003). It includes the metals/metalloids, such

as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), and zinc (Zn). Other less common metallic species that can be considered as contaminants include aluminum (Al), cesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), strontium (Sr), and uranium (U) (McIntyre 2003). Pb, one of the most persistent metals, was estimated to have a soil retention time of 150–5000 years and was reported to maintain high concentration for as long as 150 years after sludge application to soil (Nanda Kumar et al. 1995). The average biological half-life of Cd has been estimated to be about 18 years (Forstner 1995) and 10 years once in the human body (Knasmuller et al. 1998). Another concern with toxic heavy metals causing concern is that the metals may be transferred and accumulated in the body tissues of animals or human beings through food chain, which will probably cause DNA damage and carcinogenic effects by their mutagenic ability (Knasmuller et al. 1998). For example, some species of Cd, Cr, and Cu have been associated with health effects ranging from dermatitis to various types of cancer (Das et al. 1997; McLaughlin et al. 1999).

Phytoremediation, as a sustainable and inexpensive technology based on the removal of heavy metals from the environment by plants, is becoming an increasingly important objective in plant research. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of heavy metals from soils is an important goal. Arbuscular mycorrhizal fungi (AMF) provide an attractive system to advance plant-based environmental cleanup. There are two main strategies in phytoremediation, either to bind heavy metal in the soil (phytostabilization) or to import and store heavy metal in the plant's aboveground tissues (phytoextraction). AMF fungi occur in the soil of most ecosystems, including polluted soils. By acquiring phosphate, micronutrients and water and delivering a proportion to their hosts they enhance the nutritional state of their hosts. Similarly, heavy metals are taken up via the fungal hyphae and can be transported to the plant. Thus, in some cases mycorrhizal plants can show enhanced heavy metal uptake and root-to-shoot transport (phytoextraction) while in other cases AM fungi contribute to heavy metal immobilization within the soil (phytostabilization). Protection by AMF that colonise 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 (Hildebrandt et al. 2007). Even, in a study by Hildebrandt et al. (1999) it was reported that the AMF isolate *G. intraradices* Br1 consistently conferred heavy metal tolerance on a variety of plants like tomato, maize or *Medicago truncatula*, in diverse heavy metal contaminated soil under optimum fertilization. The symbiotic combination with AMF could enhance ability of hyperaccumulator plants to grow on highly contaminated soil (Hall 2002; Vogel-Mikus et al. 2006). It was also reported that AM development in hyperaccumulating species is favoured at elevated nutrient demand such as during the reproductive stage (Turnau and Mesjasz-Przybylowicz 2003; Vogel-Mikus et al. 2006).

The result of mycorrhizal colonization on clean up of contaminated soils depends on the plant–fungus–heavy metal combination and is influenced by soil conditions (Gohere and Paszkowski 2006). The degree of bioavailability of heavy metal is also a critical factor and will affect the outcome of the application (whether

revegetation and or remediation). The work of Chen et al. (2004) illustrated that well, the growth of maize (*Zea mays*) in an alkaline soil was best when the plants were colonized by *G. caledonium*. When no Zn was added to the calcareous soil, inoculated plants likely benefited from improved uptake via mycorrhizal-mediated mechanism. Moreover, at high concentrations of Zn (600 mg/kg), AMF alleviated Zn toxicity, but its translocation from roots to shoots was reduced, even with the combined use of chelating agent like EDTA. In some instances heavy metal may negatively impact the establishment of symbiosis between mycorrhiza and host plants. For example, in a study by Kim et al. (2004), it was found that the extent of colonization of *P. sylvestris* by the ecto-mycorrhiza *P. involutus* was reduced when the host plant had been previously exposed to Cd dose 10–100 mg/kg. A mycorrhizal alleviation of heavy metal toxicity to host also observed in a study by Rivera-Becerril et al. (2002). *G. intradices* alleviated the symptoms of Cd toxicity in pea (*P. sativum*). AMF enhanced soil P availability also plays an important role in metal tolerance of plant in soil. While As uptake can be fatal to plants by eventually disrupting ATP formation the presence of adequate available P in the soil has been shown to prevent cell death by competing with As for P binding sites in the Basin wildrye seeds (Knudson et al. 2003). Besides the enhanced uptake and metal binding phenomena, the mycorrhiza was also shown to change soil structure by stabilizing aggregates, thereby enhancing soil-heavy metal retention capacity (Bearden and Petersen 2000).

Audet and Charest (2007) in their study on dynamics of AM symbiosis in heavy metal phytoremediation in meta-analytical and conceptual perspectives reported that AM mediated plants accumulates greater heavy metal than non-AM plants at the low soil-heavy metal level, and the reverse at the high soil-heavy metal levels. Plant growth was greater for AM than non-AM plants at high soil-heavy metal level, whereas there were no significant differences between the AM and non-AM plants at the low to intermediated soil-heavy metal level. Although little research has been carried out on the effect of AMF on plant uptake of radionuclides, plants with mycorrhizal associations are often found more effective than non-mycorrhizal plants at the uptake of radionuclides (Entry et al. 1994). Plants inoculated with a specific mycorrhizal fungus have been shown to increase their ability to acquire necessary nutrients while removing large quantities of ^{137}Cs from soil (Varskog et al. 1994). A study done by Entry et al. (1999) included three fast growing grass species: bahia grass (*Paspalum notatum*), johnson grass (*Sorghum halpense*), and switchgrass (*Panicum virgatum*). These grasses were inoculated with two species of arbuscular mycorrhizae: *G. mosseae* and *G. intraradices* were grown in the greenhouse for 6 months. The AMF inoculated plants were found to accumulate 41.7 to 71.7% of the total ^{137}Cs that had been added to the soil while noninoculated plants accumulated 26.3 to 45.5%. The inoculated plants took up 42.0 to 88.7% of the total ^{90}Sr and the noninoculated plants only took up 23.8 to 52.6%. Plants inoculated with *G. mosseae* resulted in the highest above ground biomass and percent accumulation of both radionuclides. Recently, Goncharova (2009) reported that when plants like pea, soybean and oats were inoculated with AM, above-ground plant biomass, concentration of ^{137}Cs, above-ground plant biomass, concentration of ^{137}Cs in plant

tissue, % accumulation of ^{137}Cs from soil and the TF in all three harvests increased compared to plants growing in the control soil (not inoculated with AM).

14.5 TERI's Efforts in Reclaiming Waste Dumps and Saline Soil Using Mycorrhiza Assisted Phytoremediation Techniques

14.5.1 Case Study 1: Reclamation of Fly Ash Dykes

When coal burns, it produces fly ash-fine solid particles of ash, dust, and soot containing lead, arsenic, cadmium, cobalt, silica, mercury, and other toxic elements. Disposal of fly ash (FA) as landfill is under pressure from environmental concerns and, increasingly stringent environmental regulations are progressively increasing the cost of disposal. Many applications have been identified for fly ash. Major uses are in: cements, concrete, bricks, wood substitute products, soil stabilization, road base/embankments and consolidation of ground, land reclamation and as a soil amendment in agriculture. Despite this, a significant amount of fly ash (at least 70%) is still being disposed in lagoons and landfill. The bulk of fly ash generated in Thermal Power Plants, has been disposed of by wet and dry methods. In dry disposal, the fly ash is dumped in landfills and fly ash basins. In wet methods, the fly ash is washed out with water and piped as slurry into artificial dams, lagoons or settling ponds. This ash is often referred to as pond ash and over time the water is allowed to drain away. Both methods effectively lead to dumping of the fly ash in landfills on open land. It becomes a deadly source of health hazards when carried into the atmosphere by wind; hydrosphere and biosphere through leaching and surface run-off.

Researchers at TERI came up with the mycorrhizal mediated reclamation technology. Different strains of mycorrhizal fungi were collected from diverse regions of India and abroad. These were then isolated, selected, multiplied, and tested under greenhouse/nursery conditions to find out their growth pattern on fly ash dumps. Strains offering high tolerance, assisting in survival, and providing nutritional support to plants were selected for the purpose. With additional doses of organic and mycorrhizal fertilizer to optimize the impact, the mycorrhizal strains were then applied with selected plants on fly ash dumps. TERI has successfully established three major demonstrations of fly ash reclamation in fly ash overburdens. When put to application, life sprouted on grey, degraded, toxic wastelands in the form of green vegetation (Fig. 14.1). In a long run such green vegetation unfolds a series of physiological changes through bio-geo chemical cycle leading to enrichment of the surface and subsurface ash to promote natural ground cover (Das et al. 2013).

Mycorrhiza technology when put to application, life sprouted on grey, degraded, toxic wastelands in the form of green vegetation. In a long run such green vegetation unfolds a series of physiological changes through bio-geo chemical cycle leading to enrichment of the surface and subsurface ash to promote natural ground cover.

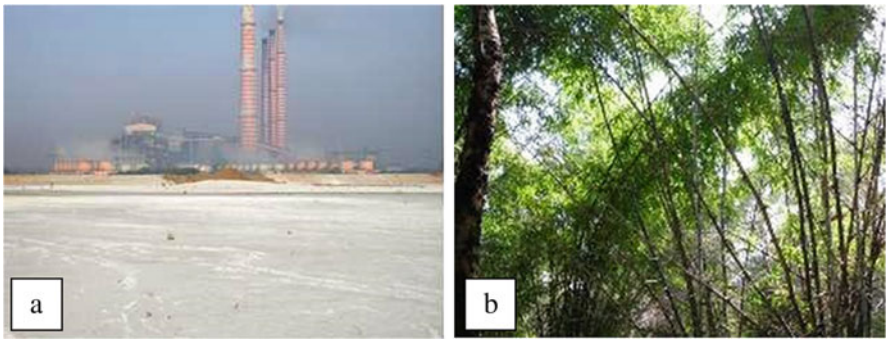


Fig. 14.1 Ash pond (**a**) Abandoned (1999) (**b**) After reclamation (2010)

14.5.2 Case Study 2: Reclamation of Chlor-alkali Sludge Laden Wasteland

The chemical wastes formed out of alkali and chloride rich sediments from industrial discharge poses a health hazard for the residents of the areas nearby. Due to high wind in coastal areas, those sludge particles blown away from the dump site to nearby residential areas causes breathing and skin problems, decolourization of clothing and even heavy corrosion of metallic structures. The site had extremely high pH and electrical conductivity and devoid of any vegetation. Moreover, sweet water scarcity in those coastal areas worsened the condition drastically. The mycorrhiza-based reclamation technologies developed by TERI becomes very relevant in this context, reclamation of these waste lands using mycorrhiza technology becomes strategically important to tackle solid wastes. TERI technology has found to be effective in development of green belt on mixed waste (chlor alkali sludge + waste from cement industry) dumps.

Despite of environmental stresses in the region, some microbes were found to sustain there. When those microbes were isolated, mass multiplied and finally applied along with mycorrhiza, they showed excellent result. Even, oil seeds, planted during earlier unsuccessful trials, started germinating. Although, initially irrigation was carried out with sweet water but after a few days, surprisingly all the plants became able to withstand sea water. Moreover, establishment of such vegetation significantly changed the physico-chemical properties of substrate such as pH, increase in organic matter, Nitrogen and Phosphorus. Significant alteration in substrate properties resulted in natural establishment of native grass species in and around the study site. The reclamation technology based on mycorrhiza organo-biofertilizer is promising enough to metamorphose the overburdens dumps into environmentally benign plantation. The reclaimed overburdens will create a positive impact in terms of people's health and environmental amelioration in addition to other social benefits such as generating large- scale employment, and revenue generation.

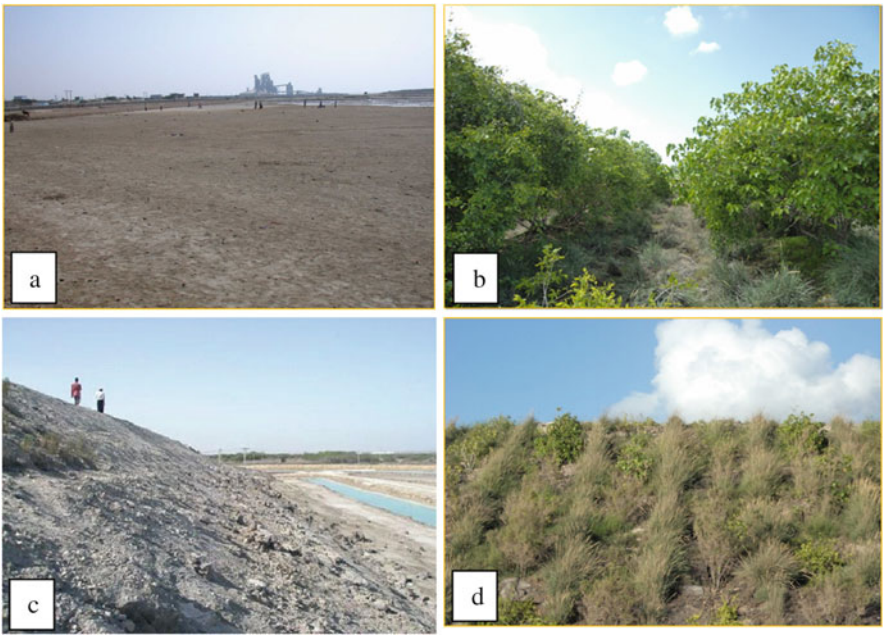


Fig. 14.2 Chlor-alkali sludge dump (**a**) Abandoned (2000) (**b**) Reclaimed (2014), Mixed waste dump (**c**) Before (2008) and (**d**) After reclamation (2014)

Over a period of 12 years, from 2002 onwards, with careful tending and experimentation, this is exactly what happened (Fig. 14.2). It started with plants sturdy enough to sustain living in the hostile substrata with only sea water for irrigation. As the plants took root, secondary growth of grasses and plants began to take place. The nature of the sediment slowly changed- it began taking on soil like qualities even in physical appearance. Over time, an ecosystem began to grow: butterflies, ants, rats, snakes, insects and a variety of small birds appeared. Moreover, establishment of such vegetation significantly changed the physico-chemical properties of substrate such as pH, increase in organic matter, N and P. Significant alteration in substrate properties resulted in natural establishment of native grass species in and around the study site.

14.5.3 Case Study 3: Reclamation of Wasteland Laden with Distillery Effluents

Distilleries generate huge amount of organic rich effluent and when it is dumped into low land areas, it causes percolation of pollutants to nearby fields, damage soil texture and health and lastly but not the least results ground water pollution. Breweries are facing such type of problem with their effluent dumpsite. Due to

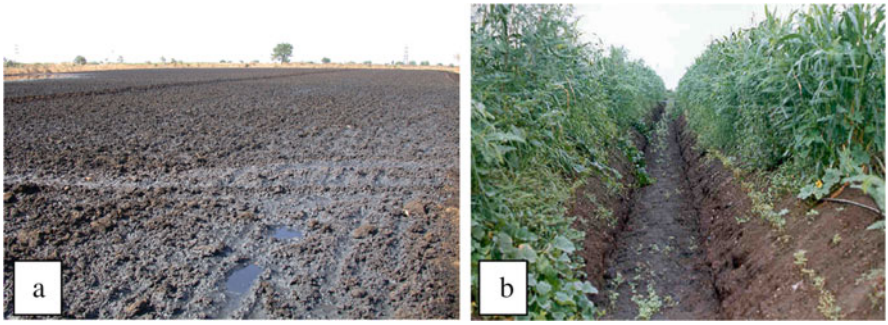


Fig. 14.3 (**a**) Distillery effluent laden land (**b**) Reclaimed land

that dumping site the color of ground water in neighboring villages got changed, during monsoon nearby lands got flooded with effluents and an intolerably foul smell from the site perpetually hung in the air. Beside that the organic sludge deposited in the dumping site had alkaline pH and very high salt content. When mycorrhiza mediated High Rate Transpiration System (HRTS) was applied to that dump site, it changed to a lush green landscape (Fig. 14.3). HRTS is a land application system where effluent from breweries is disposed in a specially designed landscape with wide ridges and furrows, and mycorrhiza mediated trees that are bestowed with high transpiration capacity. The mycorrhiza was selected on the basis of physico-chemical properties of the effluent loaded soil that was used for ridges preparation. Application of certain species of mycorrhiza along with HRTS technology made that site a commercially viable area with economic plantations.

14.5.4 Case Study 4: Production of Green Vegetable in Hyper-saline Desert Land

The hyper-saline area of Dukhan, located in the western part of Qatar has extremely adverse land and water conditions making farming difficult. Moreover, the climate is hot subtropical characterized by hot humid summers and semi short winters with scanty and infrequent rainfalls. The soil salinity level is very high and accumulation of white salts (soluble chloride and sulfates of Ca, Mg, Na and K) on soil surface (white encrustation) is a common phenomenon. When mycorrhiza based organic farming was applied to that area it changed to a productive land having plantations of tree species, ornamentals, medicinal plants, vegetables, grasses and legumes (Fig. 14.4). Beside that adaptation of such simple, farmer and eco-friendly practices resulted in enhancement of soil organic matter, improvement of pH, reduction in soluble salts (chlorides and sulfates), establishment of microenvironment for beneficial soil microbes, appearance of earthworms in plant basin and other ecosystem components such as birds, insects, snake, lizard and rabbits.

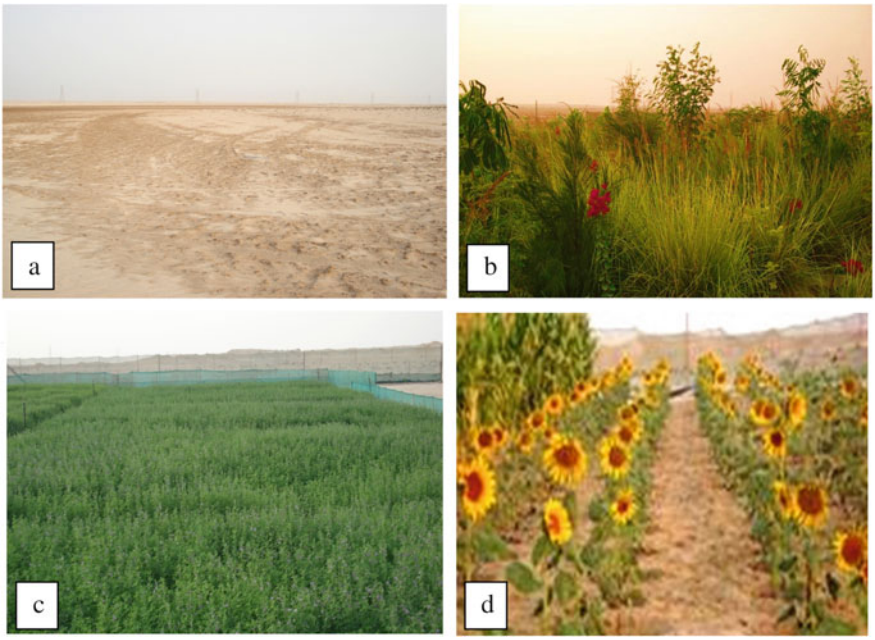


Fig. 14.4 (**a**) Hyper saline desert land (2007) (**b**) Reclaimed site (2009), Cultivation of (**c**) Fodder grass and (**d**) Sunflower

14.5.5 Case Study 5: Reclamation of Phospogypsum Waste

The Phosphogypsum (PG) is a byproduct from phosphoric acid manufacturing plants. During the production of phosphoric acid, huge amounts of solid calcium sulphate is produced as a waste by product. Solid calcium sulphate, more technically known as phosphogypsum are considered as waste material and discharged into the retention pond as slurry after mixing with sea water. Globally as well as in India, disposal of PG (open yards or stack yards) has drawn considerable attention due to likely threat to the environment (e.g. air borne dust and leachates). As expected, the phosphogypsum waste at abandoned retention pond is acidic in nature and rich in phosphate, fluoride and chlorides. Several attempts have been made using top soil, chemical fertilizer, plantation of shrubs and grasses etc. over many years but nothing could give a tangible solution to that.

The mycorrhiza-based reclamation technologies developed by The Energy and Resources Institute (TERI) for environmentally vulnerable sites becomes very relevant to tackle phosphogypsum waste to establish green belt. As expected, within a year of intervention the grey barren land get converted into lush green landscape (Fig. 14.5).

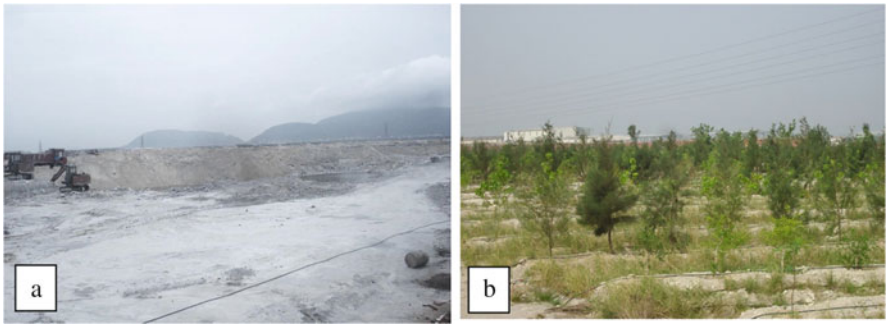


Fig. 14.5 Phospogypsum dump site (**a**) Before (2013) and (**b**) After reclamation (2014)

14.6 Conclusion

It was our intention with this chapter to draw attention to research illustrating the utility of AMF in soil remediation and its success stories with different substrates through a series of case studies and research findings. Although reports by various researchers are evident of potential role of AMF in phytoremediation, still there is a need to completely understand the ecological complexities of the plant-microbe-soil interactions and their better exploitation as consortia in remediation strategies employed for contaminated soils. To understand and explore properly, these multitrophic root microbial associations deserve multi-disciplinary investigations using molecular, biochemical, and physiological techniques.

Acknowledgement The work is the outcome of various projects and the authors duly acknowledge the financial assistance provided by National Thermal Power Corporation Limited, Korba; Vijayawada Thermal Power Station, Andhra Pradesh; Badarpur Thermal Power Station, Delhi; Technology Information and Forecasting & Assessment Council, Delhi; Fly Ash Mission, Delhi; Tata Chemicals Ltd, Gujarat; Associated Alcohols and Breweries Ltd, Madhya Pradesh; Sadeer Medical, Qatar; Coromandel International Ltd., Vizag and Department of Science and Technology, Government of India. The authors thank all workers, advisers, technicians and researchers who contributed to case studies of TERI's reclamation projects. The authors duly acknowledge the infrastructure support provided by TERI. Dr. Reena Singh is duly acknowledged for reviewing and editing the manuscript.

References

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79

Audet P, Charest C (2006) Effects of AM colonization on 'wild tobacco' plants grown in zinc-contaminated soil. Mycorrhiza 16:277–283

Audet P, Charest C (2007) Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: Meta-analytical and conceptual perspectives. Environ Pollut 147:609–614

Bafeel SO (2008) Contribution of mycorrhizae in phytoremediation of lead contaminated soils by *Eucalyptus rostrata* plants. World Appl Sci J 5:490–498

Bearden BN, Petersen L (2000) Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. Plant Soil 218:173–183

Bielinska EJ, Pranagal J (2007) Enzymatic activity of soil contaminated with triazine herbicides. Pol J Environ Stud 16:295–300

Charest C, Clark G, Dalpe Y (1997) The impact of arbuscular mycorrhizae and phosphorus on growth of two turfgrass species. J Turf Grass Manage 2:1–14

Chen B, Shen H, Li X, Feng G, Christie P (2004) Effects of EDTA application and arbuscular mycorrhizal colonization on growth and Zn uptake by maize (*Zea mays* L.) in soil experimentally contaminated with zinc. Plant Soil 261:219–229

Cheng XM, Baumgartner K (2006) Effects of mycorrhizal roots and extraradical hyphae on ^{15}N uptake from vineyard cover crop litter and the soil microbial community. Soil Biol Biochem 38:2665–2675

Das P, Samantaray S, Rout GR (1997) Studies on cadmium toxicity in plants: a review. Environ Pollut 98:29–36

Das M, Agarwal P, Singh R, Adholeya A (2013) A study of abandoned ash ponds reclaimed through green cover development. Int J Phytorem 15:320–329

Dodd JC, Burton CC, Burns RG, Jeffries P (1987) Phosphatase activity associated with the roots and the rhizosphere of plants infected with vesicular arbuscular mycorrhizal fungus. New Phytol 107:163–172

Entry JA, Rygiewicz PT, Emmingham WH (1994) Strontium-90 uptake by *Pinus ponderosa* and *Pinus radiata* seedlings inoculated with ectomycorrhizal fungi. Environ Pollut 86:201–206

Entry JA, Watrud LS, Reeves M (1999) Accumulation of cesium-137 and strontium-90 from contaminated soil by three grass species inoculated with mycorrhizal fungi. Environ Pollut 104:449–457

Forstner U (1995) Land contamination by metals: global scope and magnitude of problem. In: Allen HE, Huang CP, Bailey GW, Bowers AR (eds) Metal speciation and contamination of soil. CRC Press, Boca Raton, FL, pp 1–33

Genney DR, Alexander IJ, Killham K, Meharg AA (2004) Degradation of the polycyclic aromatic hydrocarbon (PAH) fluorene is retarded in a Scots pine ectomycorrhizosphere. New Phytol 163:641–649

Gianinazzi S, Gianinazzi-Pearson V, Tisserant B, Lemoine MC (1992) Protein activities as potential markers of functional endomycorrhiza in plants. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International Wallingford, Oxon, pp 333–339

Giri B, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmuller R, Varma A (2005) Mycorrhizosphere: strategies and functions. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Berlin, Heidelberg, pp 213–252

Gohre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122

Goncharova NV (2009) Availability of radiocasium in plant from soil: facts, mechanisms and modeling. Glob NEST J 11:260–266

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

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

Huang HL, Zhang SZ, Shan XQ, Chen BD, Zhu YG, Bell JNB (2007) Effect of arbuscular mycorrhizal fungus (*Glomus caledonium*) on the accumulation and metabolism of atrazine in maize (*Zea mays* L.) and atrazine dissipation in soil. Environ Pollut 146:452–457

Huang H, Zhang S, Wu N, Luo L, Christie P (2009) Influence of *Glomus etunicatum/Zea mays* mycorrhiza on atrazine degradation, soil phosphatase and dehydrogenase activities, and soil microbial community structure. Soil Biol Biochem 41:726–734

Jarup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182

Joner EJ, Leyval C (2003) Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza. Environ Sci Technol 37:2371–2375

Joner EJ, Johansen A, dela Cruz MAT, Szolar OJH, Loibner A, Portal JM, Leyval C (2001) Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. Environ Sci Technol 35:2773–2777

Joner EJ, Leyval C, Colpaert JV (2006) Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons (PAHs) both within and beyond the rhizosphere. Environ Pollut 142:34–38

Kazemi HV, Anderson SH, Goyne KW, Gantzer CJ (2008) Atrazine and alachlor transport in claypan soils as influenced by differential antecedent soil water content. J Environ Qual 37:1599–1607

Kim CG, Power SA, Bell JN (2004) Effects of host plant exposure to cadmium on mycorrhizal infection and soluble carbohydrate levels of *Pinus sylvestris* seedlings. Environ Pollut 131:287–294

Knasmuller S, Gottmann E, Steinkellner H, Fomin A, Pickl C, Paschke A, God R, Kundi M (1998) Detection of genotoxic effects of heavy metal contaminated soils with plant bioassay. Mutat Res 420:37–48

Knudson JA, Meikle T, DeLuca TH (2003) Role of mycorrhizal fungi and phosphorus in the Arsenic tolerance of Basin wild rye. J Environ Qual 32:2001–2006

Korade DL, Fulekar MH (2009) Development and evaluation of mycorrhiza for rhizosphere bioremediation. J Appl Biosci 17:922–929

Kothari SK, Marschner H, Romheld V (1990) Direct and indirect effects of VA mycorrhizae and rhizosphere microorganisms on mineral nutrient acquisition by maize (*Zea mays* L.) in a calcareous soil. New Phytol 116:637–645

Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. Chemosphere 50:847–853

Linderman RG (1988) Mycorrhizal interactions with the rhizosphere microflora- the mycorrhizosphere effect. Phytopathol 78:366–371

Liu SL, Luo YM, Cao ZH, LH W, Ding KQ, Christie P (2004) Degradation of benzo[a]pyrene in soil with arbuscular mycorrhizal alfalfa. Environ Geochem Health 26:285–293

Long XX, Yang XE, Ni WZ (2002) Current status and perspective on phytoremediation of heavy metal polluted soils. J Appl Ecol 13:757–762

Ma Y, Prasad MNV, Rajkumar M, Freitas H (2011) Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol Adv 29:248–258

Malcova R, Vosátka M, Gryndler M (2003) Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L. Appl Soil Ecol 23:55–67

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London

McIntyre T (2003) Phytoremediation of heavy metals from soils. Adv Biochem Eng Biotechnol 78:97–123

McLaughlin MJ, Parker DR, Clark JM (1999) Metals and micronutrients-food safety issues. Field Crops Res 60:143–163

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 emendation of Glomaceae. Mycotaxon 37:471–474

Nanda Kumar PBA, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Oliveria RS, Castro PML, Dodd JC, Vostaka M (2005) Synergistic effect of *Glomus intraradices* and *Frankia* spp. On the growth and stress recovery of *Alnus glutinosa* in an alkaline anthropogenic sediment. Chemosphere 60:1462–1470

Pawlowska TE, Charvat I (2004) Heavy-metal stress and developmental patterns of arbuscular mycorrhizal fungi. Appl Environ Microbiol 70:6643–6649

Perucei P, Scarponi L, Monotti M (1988) Interference with soil phosphatase activity by maize herbicidal treatment and incorporation of maize residues. Biol Fertil Soils 6:286–291

Purin S, Rillig MC (2008) Parasitism of arbuscular mycorrhizal fungi: reviewing the evidence. FEMS Microbiol Lett 279:8–14

Rabie GH (2004) Using wheat-mungbean plant system and arbuscular mycorrhiza to enhance in-situ bioremediation. Food Agric Environ 2:381–390

Rivera-Becerril F, Calantzis C, Turnau K, Caussanel JP, Belimov AA, Gianinazzi S, Strasser RJ, Gianinazzi P (2002) Cadmium accumulation and buffering of cadmium induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. J Exp Bot 53:1177–1185

Schoor JL (1997) Phytoremediation. Technical evaluation report for Ground-Water Remediation Technologies Analysis Center, Pittsburgh

Seibert K, Fuehr F, Cheng HH (1991) Experiments on the degradation of atrazine in the maize-rhizosphere soil. Theory and practical use of soil applied herbicide. European Weed Research Society Symposium, Paris, pp 137–146

Singh N, Megharaj M, Kookana RS, Naidu R, Sethunathan N (2004) Atrazine and simazine degradation in Pennisetum rhizosphere. Chemosphere 56:257–263

Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought stress and recovery. Physiol Plant 102:285–296

Turnau K, Mesjasz-Przybylowicz J (2003) Arbuscular mycorrhizal of *Berkheya coddii* and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa. Mycorrhiza 13:185–190

Varskog P, Naeumann R, Steinnes E (1994) Mobility and plant availability of radioactive Cs in natural soil in relation to stable Cs, other alkali elements and soil fertility. J Environ Radioact 22:43–53

Vazquez MM, Cesar S, Azcon R, Barea JM (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15:261–272

Vivas A, Marulanda A, Gomez M, Barea JM, Azcon R (2003) Physiological characteristics (SDH and ALP activities) of arbuscular mycorrhizal colonization as affected by *Bacillus thuringiensis* inoculation under two phosphorus levels. Soil Biol Biochem 35:987–996

Vogel-Mikus K, Pongrac P, Kump P, Necemer M, Regvar M (2006) Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. Environ Pollut 139:362–371

White JC, Ross DW, Gent MPN, Eitzer BD, Mattina MJI (2006) Effect of mycorrhizal fungi on the phytoextraction of weathered p,p-DDE by *Cucurbita pepo*. J Hazard Mater B 137:1750–1757

Wu NY, Zhang SZ, Huang HL, Christie P (2008a) Enhanced dissipation of phenanthrene in spiked soil by arbuscular mycorrhizal alfalfa combined with a non-ionic surfactant amendment. Science Total Environ 394:230–236

Wu NY, Zhang SZ, Huang HL, Shan XQ, Christie P, Wang YS (2008b) DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant. Environ Pollut 151:569–575

Xu SY, Chen YX, Wu WX, Wang KX, Lin Q, Liang XQ (2006) Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation. Sci Total Environ 363:206–215

Chapter 15
Conventional Methods for Mass Multiplication of AMF

Murugan Kumar and Anil Kumar Saxena

Abstract Numerous cultivation techniques have been developed in the last few decades for mass multiplication of AM fungi. Major challenges in AM fungi propagules production are the obligate nature of these fungi and non-availability of identification techniques to identify AM fungi at growth stages. Several substrates based and substrate free production techniques have been attempted for large scale production. In the present compilation we describe major conventional methods for mass multiplication of AM fungi. Different critical parameters of substrate based and substrate free techniques and the advantages and disadvantages of both the techniques have been dealt elaborately.

15.1 Introduction

Mycorrhizal fungi are ubiquitous group of fungi which are distributed worldwide, and over 90% of terrestrial plants exhibit a mutualistic symbiosis with this group of fungi forming a unique association called mycorrhizal association (Cairney 2000; Strack et al. 2003; Prasad et al. 2017). Although a wide array of mycorrhizas exist the most wide spread and ancient form of mycorrhizal association is arbuscular mycorrhiza which forms symbiosis with more than 80% of the plant species and are represented by more than 150 species of Zygomycota (Allen 1996; Strack et al. 2003). In this association the fungus acts as extension of root system taking up the role of plant's root hairs and influence beneficially to plants supporting better growth of the host plants (Garg and Chandel 2010). The various roles played by arbuscular mycorrhizal fungi (AMF) are increased mobilization and transfer of many nutrients like P, N, Zn, Fe, Cu and etc (Smith et al. 2011; Hawkins et al. 2000; Ryan and Angus 2003; Chen et al. 2004; Liu et al. 2000), production of plant growth hormones (Hause et al. 2007), increased water uptake (Wu and Xia 2006), protection of plants against soil borne pathogens through physical barrier in the root hair and secretion of antimicrobials (Pozo et al. 2002), increased overall absorption

M. Kumar • A.K. Saxena (✉)
ICAR- National Bureau of Agriculturally Important Microorgansims, Kushmaur, MauNath Bhanjan, Uttar Pradesh 275103, India
e-mail: saxena461@yahoo.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_15

capacity of roots and protection of plants against abiotic stress like drought, salt and heavy metals (Ruiz-Lozano et al. 1995; Porcel et al. 2012; Gohre and Paszkowski 2006). The multifunctional roles played by AM fungi has contributed to its recognition as inoculum in agriculture and horticulture for increasing plant growth and yield and also in forestry programs and environmental reclamation activities thereby reducing the application of agrochemicals (Schwartz et al. 2006; Rouphael et al. 2015; Urgiles et al. 2014; Moreira et al. 2016). Despite their potentials, commercial utilization of arbuscular mycorrhizal fungi is still in its infancy as compared to agrochemicals; the main reason being the obligate biotrophic nature of these AM fungi (Ijdo et al. 2011). Obligate requirement of live tissue for its multiplication means development of cost-efficient technologies for production of large quantity of high quality AM fungal inoculum is a tough task cut out for microbiologists. Conventional methods for mass multiplication of AM fungi inoculum includes substrate based production systems (nursery beds, pots, concrete tanks etc) and substrate-free (aeroponics) production systems (Mukhongo et al. 2016). Recent methods of inoculum production include root organ culture which requires sophisticated equipment's and are often costly. There are several techniques developed from time to time for mass culturing of AM fungi. The present compilation deals with conventional methods of mass multiplication of AM fungi.

15.2 Substrate Based Production of AM Fungi

In this system of mass production, large scale inoculum can be produced in pots, medium sized bags or in beds (raised or grounded), generally under controlled conditions like green houses or under more sophisticated growth chambers where one can have a say about parameters like humidity and temperature. Large scale production can also be done in open air and in field condition depending on the climate condition and host plant used for colonization. Different production strategies employed by various group of researchers for substrate based mass cultivation of AM fungi are given in Table 15.1. Important production parameters to be considered are AM fungi, host plant species in which the AM fungi is multiplied, substrates which anchor the host plants and nutrition and other amendments for the growth of both host plants and AM fungi.

15.2.1 AM Fungi

AM fungi starter inoculum is one of the most important production parameter in any substrate based production system. It generally consists of isolated spores (Tanwar and Aggarwal 2013), mixture of spores of different identified AM fungal species and mycorrhizal root pieces (Akhtar and Abdullah 2014). Wet sieving and decanting is the most widely used method to obtain individual spores (Pacioni

Table 15.1 Substrate based production of AM fungi

Plant host	AM fungi	Substrate used	Amendments	Method	Reference
Panicum maximum	*Glomus fasciculatus*	Soil and sand in 1:1 ratio	Nutrient solutions containing Urea, superphosphate and Muriate of potash	Polyenthe bags	Bagyaraj and Manjunath (1980)
Paspalum notatum	*Glomus clarum*, *Glomus mossae*, *Glomus etunicatum*, *Glomus macrocarpum*, *Gigaspora margarita*, *Gigaspora heterogama* and *Gigaspora gigantea*	Sand	Lime and Half strength Hoagland solution	Pot	Sylvia and Schenck (1983)
Manihot esculenta	*Glomus mossae*	Soil	Cassava skin with lignite slurry	Pot	Potty (1985)
Paspalum notatum	*Glomus claroideum*, *Glomus mossae*, *Glomus etunicatum*, *Glomus macrocarpum* and *Gigaspora margarita*	Soil	Sloger's nutrient solution (Slorger 1969) without phosphorus, Ammonium nitrate, calcium carbonate and potassium sulfate	Pot	Struble and Skipper (1988)
Cicer arietinum, *Arachis hypogaea*, *Zea mays* and *Pennisetum americanum*	*Glomus carum*	Sand	Hoagland's Nutrient solution	Pot	Simpson and Daft (1990)
Zea mays	*Glomus mossae* and *Glomus etunicatum*	Sand	Hoagland's Nutrient solution	Pot	Millner and Kitt (1992)
Zea mays	*Glomus intraradices*	Perlite, Sand, Vermiculite, Compost, Charcoal, Coal Marl	Hoagland's Nutrient solution	Pot	Gaur and Adholeya (2000)
Medicago sativa, *Zea mays*, *Trifolium alexondrium*, *Avena sativa* and *Sorghum vulgare*	Mixed inoculum of *Glomus*, *Gigaspora* and *Scutellospora*	Sandy loam soil	Compost	Raised beds	Gaur and Adholeya (2002)

(continued)

Table 15.1 (continued)

Plant host	AM fungi	Substrate used	Amendments	Method	Reference
Paspalum notatum	*Glomus mossae*, *Glomus etunicatum*, *Glomus claroideum*, *Glomus geosporum*, *Glomus intraradices*, *Gigaspora gigantean* and *Glomus rosea*	Vermiculite	Compost	Raised beds	Douds et al. (2005)
Hordeum vulgare, *Triticum aestivum*, *Phaseolus vulgaris* and *Phaseolus mungo*	Consortium of AM fungi containing *Glomus aggregatum*, *Glomus fasciculatum* and *Sclerocystis pakistanica*	Soil + Sand (3:1)	–	Pot	Chaurasia and Khare (2006)
Zea mays	*Glomus intraradices*	Biochar, Vermiculite, Vermicompost	Soil	Pot	Saranya and Kumutha (2011)
Zea mays, *Oryza sativa* and *Sorghum bicolor*	Mixed inoculum of *Glomus*, *Acaulospora*, *Gigaspora*, *Scutellospora* and *Sclerocystis*	Soil + Sand	–	Pot	Sadhana (2015)

1992; Tanwar and Aggarwal 2013; Selvakumar et al. 2016). Mixed inoculums are generally used for the mass multiplication of fungal species, producing intra-radical spores and vesicles (Klironomos and Hart 2002). Roots are dried and chopped into fine pieces to obtain mixture/consortium of inoculum. In addition to monospores and mixed inoculum other available options for starter inoculum are soils with mixed AM fungal spores (Gaur and Adholeya 2002) and precolonized plantlets (Douds et al. 2006). Spores used as initial inoculum are generally disinfested with streptomycin (200 ppm) or 2% chloramine T. But it is necessary to standardize the type of disinfecting agent and concentration for different AM fungi (Schenck 1982).

15.2.2 Host Plants for AM Fungi Multiplication

The most commonly used host plants for mass multiplication of AM fungi is maize (*Zea mays*). Features of a suitable host plants for AM fungi include, short life cycle, good colonization by a wide variety of AM fungi, adequate root system development, ability to grow well in low level of phosphorus, resistance to an array of stress factors like, pests, pathogens, drought and temperature extremes (Millner and Kitt 1992; Ijdo et al. 2011). A number of other plant species like *Allium* sp., *Hordeum vulgare*, *Triticum aestivum*, *Phaseolus vulgaris*, *Phaseolus mungo*, *Paspalum notatum* and others have been tried as host plant for mass multiplication of AM fungi by various researchers (Chaurasia and Khare 2006; Douds et al. 2005). Bagyaraj and Manjunath (1980) studied on the suitability of eight different grasses as host for mass multiplication of *Glomus fasciculatus* and found that *Panicum maximum* is the best host under the conditions prevailing in Bangalore India. Struble and Skipper (1988) evaluated four different host plants viz., Zea mays, *Glycine max*, *Paspalum notatum* and *Sorghum vulgare* for mass multiplication of various species of the genera *Glomus* and *Gigaspora*. They found that *Glycine max* was not a suitable host for AM fungi multiplication and gave ranking of plant species based on their suitability for mass multiplication of AM fungi. i.e., *Paspalum notatum*>*Zea mays*>*Sorghum vulgare*>*Glycine max*.

15.2.3 Substrates Anchoring Host Plants

Substrates chosen for anchoring host plants can directly influence the production of inoculum. It should contain an optimum level of nutrients in such a way that it should support the growth of host plant and at the same time induce AM fungi to sporulate and multiply (Coelho et al. 2014). The most common substrate used is soil, preferably sandy textured (Bagyaraj and Manjunath 1980; Struble and Skipper 1988; Simpson and Daft 1990; Chaurasia and Khare 2006; Saranya and Kumutha 2011; Sadhana 2015). When soil is used as substrate, wet sieving and decanting

method is followed to harvest spores and when soil is not used as a carrier for inoculum it often creates problem in harvesting and formulation (Ijdo et al. 2011). To overcome this problem many researchers started using inert materials like sand, glass beads, charcoal, perlite, biochar and vermiculite as substrates (Sylvia and Schenck 1983; Millner and Kitt 1992; Gaur and Adholeya 2000; Gryndler et al. 2003; Douds et al. 2005, 2006; Saranya and Kumutha 2011). Substrates used are generally treated to avoid contamination by undesirable microorganisms. The substrates used in closed systems like in pots and bags can be sterilized by steam or irradiation, while the substrates in raised beds can be sterilized by fumigation (Douds et al. 2005, 2006; Sadhana 2015). Some novel substrates have also been tried for AM fungi mass multiplication. Saranya and Kumutha (2011) evaluated biochar, vermiculite and vermicompost with 10% soil and without soil as a substrate for mass multiplication of *Glomus intraradices* with maize as host plants and found vermiculite +10% soil as the suitable substrate. Sugarcane baggase was used in three forms viz., fresh, dry and compost to mass multiply *Funneliformis mosseae* with onion as host. It was found that composted sugarcane baggase performed best among the three forms as substrate (Tanwar and Aggarwal 2013).

15.2.4 Nutrition and Amendments

The nutrient content of the substrate along with the added macronutrients and micronutrients influence the AM fungi directly and by inducing plant response, again influence AM fungi indirectly. Optimal nutrient content should support initial colonization, promote adequate plant growth and optimize the AM fungal propagule production (Ijdo et al. 2011). AM fungi colonization is induced by low levels of phosphorus (P) in the substrates hence, the substrates are manipulated accordingly to optimize the P status (Smith and Read 2008). Smith and Read (1997) suggested that the optimum P content for better mycorrhization and plant growth ranges from 0.01 to 0.02 mg P ml^{-1}. Generally nutrient rich soils are mixed with inert substances to optimize nutrient status of the substrate (Lee and George 2005; Douds et al. 2005, 2006; Saranya and Kumutha 2011; Sadhana 2015). When using inert material like sand as substrate nutrient solutions are added to support the growth of host plants and for multiplication of AM fungi. Nutrient solutions without P or with very less amount of P are often used to correct the nutrient deficiencies of inert materials (Sylvia and Schenck 1983; Douds and Schenck 1990; Millner and Kitt 1992; Gaur and Adholeya 2000; Lee and George 2005; Gryndler et al. 2005). In addition to nutrient solution certain amendments are also done to substrates to correct the nutrient status to optimum level. Compost and peat have been used with nutrient deficient soil (Gaur and Adholeya 2000). Organic amendments like chitin (Gryndler et al. 2003) and humic substances (Gryndler et al. 2005) have been shown to increase AM fungal colonization and hence a positive effect on mass multiplication of AM fungi, whereas cellulose was found to have negative effect on colonization (Avio and Giovannetti 1988). Application of formononetin to the

substrate increases the root colonization and density of arbuscles and vesicles (da Silva and Siqueira 1997).

15.2.5 Advantages and Disadvantages

The major advantage with the substrate based production system is that it is the least automated system and hence the cost involved is less when compared to other systems. It suits for mass multiplication of a wide array of AM fungal species either alone or as mixed inoculum (Ijdo et al. 2011). Generally substrate based production systems are convenient to produce inoculum densities of up to 80–100 propagules/cm^3 (Feldmann and Grotkass 2002). One of the major inconveniences with substrate production system is that it is not completely free from unwanted contaminants like pests and pathogens. This system of mass production consumes more space as compared to other systems. The system also warrants multiple stages of post-production purification to separate propagules from substrate or clay particles and organic debris attached to it (Millner and Kitt 1992). Separation of roots, spores and hyphae from substrate is difficult, besides there is problem of nutrient management to keep the P levels optimum for both proper growth of host and multiplication of AM fungi (Sharma et al. 2000).

15.3 Substrate-free Production of AM Fungi

Although the AM fungi inoculum is most commonly produced in solid matrixes like soil, sand or the mixture of two, inoculum can also be produced in a non-solid matrix. Production of AM fungi in non-solid matrix termed as substrate-free AM fungi production includes a wide variety of solution culture techniques (Table 15.2). These include stationary solution technique, flowing nutrient film technique (both constitutes hydroponics) and aeroponic technique (Ijdo et al. 2011). In flowing nutrient film technique plants are supported in a structure such ways that their roots come in contact with continuous flow of nutrient solution like Hoagland nutrient solution. The use of nutrient film improves gas exchange in a wider area and hence overcome the problem of deficient aeration as in the case of static culture techniques. This technique was used for inoculum production and in laboratory level mycorrhiza studies. Here the plants used for multiplication of AM fungi inoculum is either pre-colonized in a substrate based method or they become mycorrhizal after they are introduced into the apparatus (Howeler et al. 1981, 1982a, b; Mosse and Thompson 1984; Elmes and Mosse 1984). Stationary solution technique also uses low concentration of nutrient solution and is similar to the nutrient film technique except for the fact that here the nutrient solution is frequently aerated. Here continuous aeration is avoided to curb the damages to delicate hyphae caused by strong movement of nutrient solution (Crush and Hay 1981;

Table 15.2 Substrate free production of AM fungi

Plant host	AM fungi	Method	Reference
Maninot esculenta	AM fungi*	Flowing nutrient film technique	Howeler et al. (1981)
Maninot esculenta, Oryza sativa, Zea mays, Vigna unguiculata, Phaseolus vulgaris	AM fungi*	Flowing nutrient film technique	Howeler et al. (1982a, b)
Phaseolus vulgaris	*Glomus mosseae, Gomus fasciculatum*	Flowing nutrient film technique	Mosse and Thompson (1984)
Zea mays	*Glomus mosseae*	Flowing nutrient film technique	Elmes and Mosse (1984)
Trifolium repens	*Gigaspora margarita*	Stationary solution culture technique	Crush and Hay (1981)
Linum usitatissimum, Paspalum notatum	*Glomus intraradices*	Stationary solution culture technique	Dugassa et al. (1995)
Triticum aestivum, Sorghum bicolor	*Glomus mosseae*	Stationary solution culture technique	Hawkins and George (1997)
Ipomoea batatas, Paspalum notatum	*Glomus deserticola, Glomus etunicatum, Glomus intraradices, Glomus etunicatum, Glomus intraradices*	Aeroponic using home humidifier for propelling nutrient solution on the root surface	Hung and Sylvia (1988)
Sorghum sudanense	*Glomus intraradices*	Aeroponic using Atomizing disc and Ultrasonic nebulizer	Mohammad et al. (2000)

* indicates a mixture of AM fungi is used for mass production

Dugassa et al. 1995; Hawkins and George 1997). These two techniques; stationary solution technique and flowing nutrient film technique constitutes hydroponics techniques for AM fungi inoculum production.

Another form of substrate-free production system exists, called aeroponics wherein the plant nutrients are supported in a structure and roots are continuously bathed using nutrient solution mist in a closed chamber (Jasper et al. 1979). Aeration is improved by spraying of microdroplets of nutrient solution thereby allowing increased gas exchange in the roots (Ijdo et al. 2011). The mist can be

applied through various aeroponic devices like atomizing disk, micro irrigated nozzle and an ultrasonically generated fog. Jarstfer and Sylvia (1995) reported the nozzle spray system was the best for AM fungi inoculum production, while in another study it was reported that ultrasonic method was the best for inoculum production (Mohammad et al. 2000). Inoculum production of AM fungi in aeroponic culture has many advantages like easy extraction of inoculum (spores, hyphae or infectious roots) and the roots may be sheared to produce inoculum that has high spore count and efficient to handle (Sylvia and Jarstfer 1992a, b).

Various production parameters to be considered in substrate free production systems are AM fungi, host plants, nutrition and other additional factors.

15.3.1 AM Fungi

In the initial studies on substrate free production system not much of importance was given to the identity of AM fungi, for instance Howeler et al. (1981, 1982a, b) used nutrient film technique for production of AM fungi inoculum employing different host plant in which there was not even a mention on the species of AM fungi used for inoculum production. But with the improvement in the taxonomy of AM fungi researchers started giving importance to the species of AM fungi for production of inoculum. Several species of AM fungi have been used to set up substrate-free inoculum production. *Glomus mosseae*, *Gomus fasciculatum*, *Gigaspora margarita*, *Glomus intraradices* and *Acaulospora levis* have been used to produce incolum by both nutrient film technique and stationary solution culture technique (Mosse and Thompson 1984; Elmes and Mosse 1984; Crush and Hay 1981; Dugassa et al. 1995; Hawkins and George 1997). *Glomus deserticola*, *Glomus etunicatum*, *Glomus intraradices*, *Glomus etunicatum*, *Glomus intraradices* and *Entrophosphora kentinensis* have been used to produce inoculum employing aeroponic technique (Hung and Sylvia 1988; Wu et al. 1995; Mohammad et al. 2000, 2004). Tajini et al. (2009) reported the suitability of *Glomus intraradices* which has grown successfully in a hydroaeroponic system in what is called tripartite hydroaeroponic system. The study also reported the failure *Glomus rosea* to grow using this system but there was not any attempt to find out why *G. intraradices* grew and *G. rosea* failed to grow.

15.3.2 Host Plants for AM Fungi Multiplication

Several host plants used in the substrate based production system have been found suitable in substrate production system also. For nutrient film technique of hydroponic system, plants tried as host are *Maninot esculenta*, *Oryza sativa*, *Zea mays*, *Vigna unguiculata*, *Phaseolus vulgaris* and *Zea mays* (Howeler et al. 1981; 1982a, b; Mosse and Thompson 1984; Elmes and Mosse 1984). *Triticum aestivum*,

Trifolium repens, *Sorghum bicolor* and *Linum usitatissimum* have been used successfully for static system of hydroponics (Crush and Hay 1981; Dugassa et al. 1995; Hawkins and George 1997). Among the plants suggested for aeroponics are *Ipomoea batatas, Paspalum notatum* and *Sorghum sudanense* (Hung and Sylvia 1988; Wu et al. 1995; Jarstfer and Sylvia 1995; Mohammad et al. 2000). Colonization level of AM fungi may be influenced by the choice of host plants and the nutrient requirement of any production system may vary with the combination of type of host plants and AM fungi species (Ijdo et al. 2011).

15.3.3 Nutrition

Major considerations in this parameter are better quality water and concentration of nutrients solution. Deionized or distilled water is often recommended to use considering the pH, buffering capacity of nutrient solution which should be free from any micronutrient in potentially toxic concentrations. As the roots are in direct contact with nutrient solution the concentration of nutrient solution should be optimum (Jarstfer and Sylvia 1995). Existing nutrient solutions like Knop's, Hoagland's and Long Ashton were used as such or with some modifications in many studies of substrate free production system (Ijdo et al. 2011). As in the case of substrate based production system concentration of P is crucial in the nutrient solution (Elmes and Mosse 1984; Jarstfer and Sylvia 1995; Hawkins and George 1997). Hawkins and George (1997) recommended a phosphorus concentration of 1–50 μM, a concentration found in natural soil solution. But in general a concentration of 0.5–10 μM available P is often used by various groups of researchers (Ijdo et al. 2011). Overall concentration of nutrient solution as recommended by Jarstfer and Sylvia (1997) is <1–24 μM. Though there is a generalization of P concentration optimum concentration of nutrient solution have to be optimized depending on the combination of host plants and AM fungi species.

15.3.4 Additional Factors

The other additional factors to be considered are pH of the nutrient solution, temperature and relative humidity. Elmes and Mosse (1984) have found the pH in the range of 6.5 to 7.2 as optimum for hydroponic system. Jarstfer and Sylvia (1995) maintained a pH near neutral and temperature between 15 °C and 35 °C for optimum colonization of AM fungi. Superficial illumination was given to plants during cultivation for proper growth and hence better colonization by AM fungi (Jarstfer et al. 1988; Jarstfer and Sylvia 1995; Hawkins and George 1997; Mohammad et al. 2000; Tajini et al. 2009). It is proposed to use adequate wavelength (400–700 nm) and high photosynthetic photon flux density (Jarstfer and Sylvia 1997). Hawkins and George (1997) used a relative humidity of 60%. Hung

and Sylvia (1988) have maintained a temperature range of 24–35 °C and photosynthetic photon flux density of 1725–1850 $\mu Em^{-2}S^{-2}$ for mass multiplication of *Glomus deserticola* and *Glomus etunicatum* with *Paspalum notatum* as host plant. Martin-Laurent et al. (1999) maintained a temperature of 26–28 °C, high relative humidity of 80–850% and high illuminance of 550–1100 $\mu Em^{-2}S^{-2}$ for aeroponic production of *Acacia mangium* saplings associated with AM fungi. Though several workers have used varying level of pH, temperature, RH and other factors, additional works have to carried out to study the effect of pH, RH, temperature and other plant growth regulators.

15.4 Conclusion

Mass multiplication of AM fungi is a prerequisite to fundamental research as well as for application purposes. There are numerous custom-made methods available for mass production of AM fungi for lab-scale and large fields. With the present level of technology AM fungi propagules cannot be produced without a host. Further the species of AM fungi cannot be identified in their active stages. This creates a problem with quality control. In the present scenario, hydroponics, aeroponics and nutrient flow techniques are the most widely used production technologies under conventional method of mass multiplication of AM fungi. Among these techniques aeroponics is most appropriate technique for production of pure strain of AM fungi. The major risk involved in inoculating AM fungi in the field is the inoculation of unwanted microorganisms along with AM fungi when production techniques are inferior where along with AM fungi unwanted microorganisms are also mass produced unintentionally. Such introduction of undesirable microorganisms can be avoided only when mass production is done under strict in vitro conditions. Root organ culture is one such in vitro production technology but it does not come without high cost of production. We are in an era where inoculation of other beneficial microorganisms outcompete AM fungi inoculum primarily due to difficulties involved in mass cultivation of AM fungi. AM fungi inoculation are often more beneficial as compared to other beneficial microorganisms. But having said that, to realize the actual potential of AM fungi mass production techniques should be economically feasible with technical ease.

References

Akhtar MS, Abdullah SNA (2014) Mass production techniques of arbuscular mycorrhizal fungi: major advantages and disadvantages: a review. Biosci Biotechnol Res Asia 11:1199–1204

Allen MF (1996) The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. Mycol Res 100:769–782

Avio L, Giovannetti M (1988) Vesicular-arbuscular mycorrhizal infection of lucerne roots in a cellulose-amended soil. Plant Soil 112:99–104

Bagyaraj DJ, Manjunath A (1980) Selection of a suitable host for mass production of VA mycorrhizal inoculum. Plant Soil 55:495–498

Cairney JWG (2000) Evolution of mycorrhiza systems. Naturwissenschaften 87:467–475

Chaurasia B, Khare PK (2006) *Hordeum vulgare*: a suitable host for mass production of arbuscular mycorrhizal fungi from natural soil. Appl Ecol Environ Res 4:45–53

Chen B, Shen H, Li X, Feng G, Christie P (2004) Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (*Zea mays* L.) in soil experimentally contaminated with zinc. Plant Soil 261:219–229

Coelho IR, Pedone-Bonfim MV, Silva FS, Maia LC (2014) Optimization of the production of mycorrhizal inoculum on substrate with organic fertilizer. Braz J Microbiol 45:1173–1178

Crush JR, Hay MJM (1981) A technique for growing mycorrhizal clover in solution culture. New Zeal J Agr Res 24:371–372

da Silva JP Jr, Siqueira JO (1997) Application of synthetic formononetin to the soil as a stimulant of mycorrhizal formation in maize and soybean. Revista Brasileira de Fisiologia Vegetal 9:135–141

Douds DD Jr, Schenck NC (1990) Increased sporulation of vesicular–arbuscular mycorrhizal fungi by manipulation of nutrient regimens. Appl Environ Microbiol 56:413–418

Douds DD Jr, Nagahashi G, Pfeffer PE, Kayser WM, Reider C (2005) On-farm production and utilization of arbuscular mycorrhizal fungus inoculum. Can J Pl 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

Dugassa DG, Grunewaldt-Stocker G, Schonbeck F (1995) Growth of *Glomus intraradices* and its effect on linseed (*Linum usitatissimum* L.) in hydroponic culture. Mycorrhiza 5:279–282

Elmes RP, Mosse B (1984) Vesicular-arbuscular endomycorrhizal inoculum production II Experiments with maize (*Zea mays*) and other hosts in nutrient flow culture. Can J Bot 62:1531–1536

Feldmann F, Grotkass C (2002) Directed inoculum production- shall be able to design AMF populations to achieve predictable symbiotic effectiveness? In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhauser, Basel, pp 261–279

Garg N, Chandel S (2010) Arbuscular mycorrhizal networks: process and functions: a review. Agron Sustain Devel 30:581–599

Gaur A, Adholeya A (2000) Effects of particle size of soil-less substrates upon AM fungus inoculum production. Mycorrhiza 10:43–48

Gaur A, Adholeya A (2002) Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. Biol Fert Soils 35:214–218

Gohre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122

Gryndler M, Jansa J, Hrselova H, Chvatalova I, Vosatka M (2003) Chitin stimulates development and sporulation of arbuscular mycorrhizal fungi. Appl Soil Ecol 22:283–287

Gryndler M, Hrselova H, Sudova R, Gryndlerova H, Rezacova V, Merhautova V (2005) Hyphal growth and mycorrhiza formation by the arbuscular mycorrhizal fungus *Glomus claroideum* BEG 23 is stimulated by humic substances. Mycorrhiza 15:483–488

Hause B, Mrosk C, Isayenkov S, Strack D (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68:101–110

Hawkins HJ, George E (1997) Hydroponic culture of the mycorrhizal fungus *Glomus mosseae* with *Linum usitatissimum* L., *Sorghum bicolor* L. and *Triticum aestivum* L. Plant Soil 196:143–149

Hawkins HJ, Johansen A, George E (2000) Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226:275–285

Howeler RH, Edwards DG, Asher CJ (1981) Application of flowing solution culture techniques to studies involving mycorrhizas. Plant Soil 59:179–183

Howeler RH, Cadavid LF, Burckhardt E (1982a) Response of cassava to VA mycorrhizal inoculation and phosphorus application in greenhouse and field experiments. Plant Soil 69:327–339

Howeler RH, Edwards DG, Asher CJ (1982b) Establishment of an effective endomycorrhizal association on cassava in flowing solution culture and its effects on phosphorus nutrition. New Phytol 90:229–238

Hung LLL, Sylvia DM (1988) Production of vesicular-arbuscular mycorrhizal fungus inoculum in aeroponic culture. Appl Environ Microbiol 54:353–357

Ijdo M, Cranenbrouck S, Declerck S (2011) Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21:1–16

Jarstfer AG, Sylvia DM (1995) Aeroponic culture of VAM fungi. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Heidelberg, pp 427–441

Jarstfer AG, Sylvia DM (1997) Isolation, culture and detection of arbuscular mycorrhizal fungi. In: Hurst CJ (ed) Manual of environmental microbiology. American Society of Microbiology, Washington, DC, pp 406–412

Jarstfer AG, Farmer-Koppenol SDM, Sylvia DM (1988) Tissue magnesium and calcium affect arbuscular mycorrhiza development and fungal reproduction. Mycorrhiza 7:237–342

Jasper DA, Robson AD, Abbot LK (1979) Phosphorus and the formation of vescicular-asbuscular mycorrhizas. Soil Biol Biochem 11:501–505

Klironomos JN, Hart MM (2002) Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184

Lee YJ, George E (2005) Development of a nutrient film technique culture system for arbuscular mycorrhizal plants. Hortic Sci 40:378–380

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Martin-Laurent F, Lee SK, Tham FY, Jie H, Diem HG (1999) Aerponics production of *Acacia mangium* saplings inoculated with AM fungi for reforestation in the tropics. Forest Ecol Manag 122:199–207

Millner PD, Kitt DG (1992) The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. Mycorrhiza 2:9–15

Mohammad A, Khan AG, Kuek C (2000) Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi. Mycorrhiza 9:337–339

Mohammad A, Mirta B, Khan AG (2004) Effects of sheared-root inoculum of *Glomus intraradices* on wheat grown at different phosphorus levels in the field. Agric Ecosyst Environ 103:245–249

Moreira H, Pereira SI, Marques AP, Rangel AO, Castro PM (2016) Mine land valorization through energy maize production enhanced by the application of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi. Environ Sci Pollut Res 23:6940–6950

Mosse B, Thompson JP (1984) Vesicular-arbuscular endomycorrhizal inoculum production. I. Exploratory experiments with beans (*Phaseolus vulgaris*) in nutrient flow culture. Can J Bot 62:1523–1530

Mukhongo RW, Tumuhairwe JB, Ebanyat P, AbdelgGadir AH, Thuita M, Masso C (2016) Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan Africa: challenges and ways of improving. Int J Soil Sci 11:108–122

Pacioni G (1992) Wet-sieving and decanting techniques for the extraction of spores of vesicular-arbuscular fungi. Method Microbiol 24:317–322

Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sustain Dev 32:181–200

Potty VP (1985) Cassava as an alternate host for multiplication of VAM fungi. Plant Soil 88:135–137

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. J Exp Bot 53:525–534

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 - Function, Diversity, State of the Art. Springer International publishing AG, pp 1–7

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Colla G (2015) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci Hortic 196:91–108

Ruiz-Lozano JM, Azcon R, Gomez M (1995) Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Appl Environ Microbiol 61:456–460

Ryan MH, Angus JF (2003) Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. Plant Soil 250:225–239

Sadhana B (2015) Mass production of AM fungal inoculum by soil based pot culture. Int J Adv Res Bio Sci 2:129–133

Saranya K, Kumutha K (2011) Standardization of the substrate material for large scale production of arbuscular mycorrhizal inoculum. Int J Agric Sci 3:71–77

Schenck NC (1982) Methods and principles of mycorrhizal research. American Phytopathological Society, St Paul, MN

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

Selvakumar G, Krishnamoorthy R, Kim K, Sa T (2016) Propagation technique of arbuscular mycorrhizal fungi isolated from coastal reclamation land. Eur J Soil Biol 74:39–44

Sharma AK, Singh C, Akhauri P (2000) Mass culture of arbuscular mycorrhizal fungi and their role in biotechnology. Proc Ind Nat Sci Acad B66:223–238

Simpson D, Daft MJ (1990) Spore production and mycorrhizal development in various tropical crop hosts infected with *Glomus clarum*. Plant Soil 121:171–178

Smith SE, Read DJ (1997) Vesicular-arbuscular mycorrhizas in agriculture and horticulture. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis. Academic Press, London, pp 453–469

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Smith SE, Jakobsen I, Gronlund 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

Strack D, Fester T, Hause B, Schliemann W, Walter MH (2003) Review paper: arbuscular mycorrhiza: biological, chemical, and molecular aspects. J Chem Ecol 29:1955–1979

Struble JE, Skipper HD (1988) Vesicular-arbuscular mycorrhizal fungal spore production as influenced by plant species. Plant Soil 109:277–280

Sylvia DM, Jarstfer AG (1992a) Sheared roots as a VA-mycorrhizal inoculum and methods for enhancing growth. US Patent 7, 574, 763 17 Mar 1992

Sylvia DM, Jarstfer AG (1992b) Sheared-root inocula of vesicular-arbuscular mycorrhizal fungi. Appl Environ Microbiol 58:229–232

Sylvia DM, Schenck NC (1983) Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus tolerant vesicular-arbuscular mycorrhizal fungi. New Phytol 95:655–661

Tajini F, Suriyakup P, Vailhe H, Jansa J, Drevon JJ (2009) Assess suitability of hydroaeroponic culture to establish tripartite symbiosis between different AMF species, beans, and rhizobia. BMC Plant Biol 9:73. https://doi.org/10.1186/1471-2229-9-73

Tanwar A, Aggarwal A (2013) Sugarcane Bagasse: a novel substrate for mass multiplication of *Funneliformis mosseae* with onion as host. J Cent Eur Agric 14(4):1502–1511

Urgiles N, Strauß A, Lojan P, Schußler A (2014) Cultured arbuscular mycorrhizal fungi and native soil inocula improve seedling development of two pioneer trees in the Andean region. New Forests 45:859–874

Wu QS, Xia RX (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425

Wu CG, Liu YS, Hung LL (1995) Spore development of *Entrophospora kentinensis* in an aeroponic system. Mycologia 87:582–587

Chapter 16
Biological Hardening of Micropropagated Tomato Plantlets: A Case Study with *Piriformospora indica*

RK Gupta, VS Verma, Anil Bhushan, and Vijay Raina

Abstract Aseptic cultures were established from explants (cotyledons, shoot-tips and hypocotyls) in six genotypes (two hybrids—TH802 & TH2312; respective parents Haelani × Accession-2 and VFN8 × Punjab Chuhara) and three male sterile (*ms*) lines (EC251735, EC 251692 and EC 251752) in tomato (*Solanum lycopersicum* L.) on MS medium with different concentrations and combinations of hormones. Maximum number of shoots in six genotypes initiated on cotyledons as explants on MS medium supplemented with BAP (2.0 mg/l) while only three male sterile lines initiated on medium with BAP (0.5 mg/l) + Kinetin (0.5 mg/l). Multiple shoot formation on subsequent subcultures was observed on medium with BAP (1 mg/l) + Kinetin (0.5 mg/l). The separated shoots (4–5 cm) in hybrids/parents/*ms* lines resulted in profuse rooting on half basal MS medium (0.5× MS media) containing 15 g l^{-1} sucrose (without hormones). Hardening of in vitro raised plantlets in hybrids/parents/*ms* lines was done using bioagents and antitranspirants. Maximum survival was recorded on treatment of plantlets with *Piriformospora indica* (86.6%) followed by *Trichoderma viride* (75.0%), while minimum survival was observed in untreated control (36.6%). The hardened plantlets were successfully transferred to field and expressed true-to-type traits (including male sterility). This paper reports an efficient and reproducible protocol for in vitro multiplication and biological hardening of diverse genotypes in tomato.

R. Gupta (✉) • A. Bhushan • V. Raina
Division of Vegetable Science and Floriculture, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu 180009, Jammu and Kashmir, India
e-mail: profrkgupta@rediffmail.com

V. Verma
Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu 180009, Jammu and Kashmir, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_16

16.1 Introduction

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae, which contains more than 3000 species. Tomato was introduced to Europe in the fifteenth century and in the Indian subcontinent about 200 years ago. It has now become one of the most popular vegetable crops of the world. Its genetics is well studied and genome mapped. *S. lycopersicum cerasiforme* was considered as an ancestor of the cultivated tomato because of its abundant existence in Central America (Bai and Lindhout 2007). Recent studies have revealed that the closest relative of the cultivated tomato is *S. pimpinellifolium* (The Tomato Genome Consortium 2012). In short history of tomato, hybrid and transgenic cultivars have now been developed for higher yield and other useful traits. Traditionally, production of hybrid seed involves hand emasculation and pollination leading to high cost of seeds, while male sterility has not been exploited because of difficulty of maintenance of *ms* lines. Plant tissue culture techniques that help plant breeders include micropropagation for rapid multiplication, embryo-rescue for wide hybridization, somatic hybridization to combine non-crossable species and transgenic to introduce genes from other organisms. Plant tissue culture also holds potential for multiplication of true-to-type and virus-free planting material and genetic transformation in tomato. But the stunted growth/poor survival of in vitro raised plantlets upon direct transfer to ex vitro conditions or in the field (without hardening) has hampered the progress due to prevalence of relatively lower humidity, higher light intensity and harmful soil microorganisms and/or inefficient stomata, weak root system and poorly developed cuticle.

At the same time, antitranspirants and biopriming have shown varying degrees of success in improving ex vitro survival or performance. The leaf-surface covering agents such as glycerol, paraffin and grease have been used to promote ex vitro survival in many herbaceous plant species (Selvapandiyan et al. 1988). Arbuscular mycorrhizal fungi (AM) has also been used for biological hardening of micropropagated plantlets, but lack of an authentic AMF axenic culture limits its applications. *P. indica*—a root endophyte resembles AMF in morphology, physiology and mode of inter- and intracellular invasion of the mycelium to the cortical region of the root (Prasad et al. 2005), because of ease of culture and plant growth promotional effect. *P. indica* has been used for hardening of micropropagated plantlets in various crops (Sahay and Varma 1999, 2001; Varma et al. 1999, 2001, 2012; Prasad et al. 2013).

Development of efficient micropropagation and hardening protocol has opened new vistas for multiplication of disease-free planting material in hybrids or that of male sterile lines for further use in hybrid seed production (Bhushan and Gupta 2010). Plantlets are usually developed in vitro on Murashige and Skoog (MS) medium under low light intensity and high humidity levels require acclimatization or hardening, both under in vitro and ex vitro conditions for improving survival and establishment in field. In this chapter, we report a case study on micropropagation of tomato hybrids/*ms* lines and hardening using glycerol, ABA and *P. indica* in different treatment combinations for true-to-type and disease -free multiplication plantlets in tomato.

The technique in which propagules are cloned from tissue (small explants) taken from a single plant is known as micro-propagation. During multiplication phase, mostly, the initiated shoots are in clusters and these are separated and roots induced. After plantlets have been developed, various steps are taken to prepare them for adaptation to the external environment. In order to boost shoot and root development, the individual plantlets are transferred to a standardized medium or potting mixture for 3–4 weeks. The utmost care is taken in moving plantlets from sterile and artificially controlled environment to gradually increasing light intensity and reducing humidity for the purpose of acclimatization to facilitate successful field transfer of in vitro raised plantlets.

The various studies on in vitro multiplication of tomato often display problems concerning survival and establishment on direct transfer from in vitro conditions to pots or in field under open environment. The plantlets developed in vitro from hybrids/*ms* lines under low light intensity and high humidity levels are exposed to minimal stress and require acclimatization (physical/chemical/biological) both under in vitro and ex vitro conditions. Attempts to multiply disease-free planting material of tomato hybrids/*ms* lines using explants (cotyledon, shoot tip, leaf and hypocotyls) in vitro and hardening by our group have revealed some useful information.

16.2 Protocol

The seeds of two tomato hybrids (TH802 and TH2312) with their respective parents (Haelani × Accession-2 and VFN8 × Punjab Chuhara) were obtained from the Department of Vegetable Crops, PAU, Ludhiana, India and three *ms* lines (EC 251735, EC251692 and EC251752) from NBPGR, New Delhi, India. MS medium (Murashige and Skoog 1962) was prepared and adjusted to pH 5.8, filled into culture bottles and sterilized by autoclaving at 15 psi for 20 min.

16.2.1 Seed Sterilization and in Vitro Germination

After testing different combinations, for optimum sterilization, tomato seeds were surface sterilized by quick dip (30s) in 70% ethanol (v/v) followed by treatment with 4.0% NaOCl for 3 min and then washed three times with sterilized distilled water. The seeds were inoculated on MS medium with 30 g/L sucrose and 8.0 g/L agar (pH 5.8) in culture tubes or Petri dishes sealed and kept in dark for 3 days at 25±2 °C. Subsequently, these cultures with germinating seeds were kept in culture room on the same temperature with fluorescent light and 16 h light/8 h dark photoperiod for 10–14 days.

16.2.2 Establishment of Aseptic Cultures and Shoot Initiation

About 2-week old in vitro grown seedlings served as source of explants (cotyledons, leaf, shoot tips, hypocotyls). The explants excised under aseptic conditions were put on MS medium supplemented with different concentrations and combinations of Benzyl Amino Purine (BAP) and Kinetin. These cultures were maintained in culture room with around 1500–2000 lux fluorescent light and 16 h photoperiod and maintained at 25 ± 2 °C temperature. The percentage response towards shoot emergence/initiation was recorded after 4 weeks, while that of shoots per culture after second subculture.

16.2.3 Maintenance/Multiplication of Shoots and Rooting

The regenerated shoots obtained after 4 week of first sub-culture were maintained for multiplication on MS medium fortified with 2 mg/l BAP for hybrids and 0.5 mg l^{-1} BAP and 0.5 mg/l Kinetin for *ms* lines. Rooting in regenerated shoots was induced on basal MS medium (without hormone) solidified with agar-agar (6 gl^{-1}) after about 10–15 days in hybrids or ½ basal MS medium (without hormone) in *ms* lines.

16.2.4 Hardening/Acclimatization

The plantlets were transferred to small plastic pots containing potting mixture (sand, soil and FYM in ratio of 1: 1: 1) fortified with *T. viride*. The *P. indica* cultures with the help of loop were applied at the base of the stem and pots kept for about 10 days near window in the room with indirect sunlight and then transferred to bigger pots or field.

16.3 Essential Items Needed

16.3.1 In Vitro Hardening of Plantlets

Before transfer of plantlets from the cultured vessels to small plastic pots, the caps or tops of the culture vessels containing growing plantlets were removed and cultures kept in indirect natural light near windows in closed rooms or Greenhouse for a period 7–10 days for the purpose of in vitro acclimatization. In vitro rooted pre-hardened plantlets obtained from the culture vessels were gently washed to

remove the adhering medium from the roots so as to avoid the growth of disease-causing micro-organisms. After washing thoroughly under running tap water, plantlets were left in the tray full of water or placed over cotton soaked in water under high light conditions for 3–4 h. These plantlets were then used for ex vitro hardening.

16.3.2 Ex Vitro Hardening of Plantlets

In another experiment, different treatment combinations of chemicals with biological agents were tested for *ms* lines. Each treatment combination contained 60 plants. The plant survival was calculated after 1 and 3 weeks and observations on agro-biological features were recorded at various growth and maturity stages.

16.3.3 Antitranspirants: Preparation and Use

The stem and leaves of micropropagated plantlets were dipped in solution of glycerol (0.5%) and ABA (7 ppm) for 5 min and transferred to small plastic pots kept in green house and then transferred to bigger pots or field.

16.3.4 Piriformospora indica: Preparation and Use

The culture of *P. indica* was obtained from Ajit Varma of Jawaharlal Nehru University, New Delhi, India and maintained and multiplied in a culture bottle or Petri plate containing minimal agar medium (pH 4.8) and incubated for 7 days at 30 °C in dark (Prasad et al. 2005). Further multiplication of *P. indica* using one small agar disc (about 1 cm in diameter) loaded with hyphae and spores and placing the same in a Petri plate (90 mm containing modified minimal agar medium). A layer of *P. indica* inoculum was either mixed in a small amount of fine sterile soil or compost and then placed as a top layer in small plastic pots containing potting mixture and the plantlets were transferred. The inoculum was added at a rate of 1% of the total fine compost in the pot (w/v). Alternatively, the inoculum was kept near base of the stem of the transferred plantlet in small plastic pot under controlled conditions.

16.3.5 Trichoderma viride: Preparation and Use

The cultures of *T. viride* were obtained from Indian Institute of Integrative Medicine (CSIR), Jammu. The sterilized potting mixture (Soil: Sand: FYM ::1:1:1) was treated with *T. viride* powder @ 2 g/Kg of pot mixture and then poured into small

plastic pots where in vitro raised plants were transferred and kept under controlled conditions.

The pre-hardened plantlets were then transferred to small plastic cups containing sterilized potting mixture directly or after treatment with chemicals (Glycerol 0.5% and ABA 5 ppm) and/or biological hardening agents using nine combinations with detail as under:

T_1 = *T. viride* (2 g/Kg of soil mix)
T_2 = *P. indica* (one slice per plant)
T_3 = ABA (7 ppm)
T_4 = *T. viride* (2 g/Kg of soil mix) + ABA (7 ppm)
T_5 = *P. indica* (one slice/plant) + ABA (7 ppm)
T_6 = Glycerol (0.5%)
T_7 = *T. viride* (2 g/kg of soil mix) + Glycerol (0.5%)
T_8 = *P. indica* (one slice/plant) + Glycerol (0.5%)
T_9 = Control (untreated)

The small plastic pots containing plantlets under different treatment combinations were kept initially near window in the room and later transferred to greenhouse with partial shading or reduced light by 50% using shade net or hardening facility and watered with 20–50 ml of 0.5× MS salts solution. These plantlets were gradually exposed to increasing light intensity and reducing humidity for 3–4 weeks and then transferred to bigger pots or in the field in full sun.

16.4 Observations and Interpretations

Efficient and easily reproducible micropropagation protocol and hardening procedure for hybrids and *ms* lines developed in our laboratory have been given in Figs. 16.1 and 16.2.

16.4.1 Micropropagation

The cultures were initially established from explants (cotyledon, shoot-tip and hypocotyl) in six genotypes (two hybrids—TH802 & TH2312; respective parents Haelani × Accession-2 and VFN8 × Punjab Chuhara) and three *ms* lines (EC251735, EC 251692 and EC 251752) using MS medium with different concentrations and combinations of hormones.

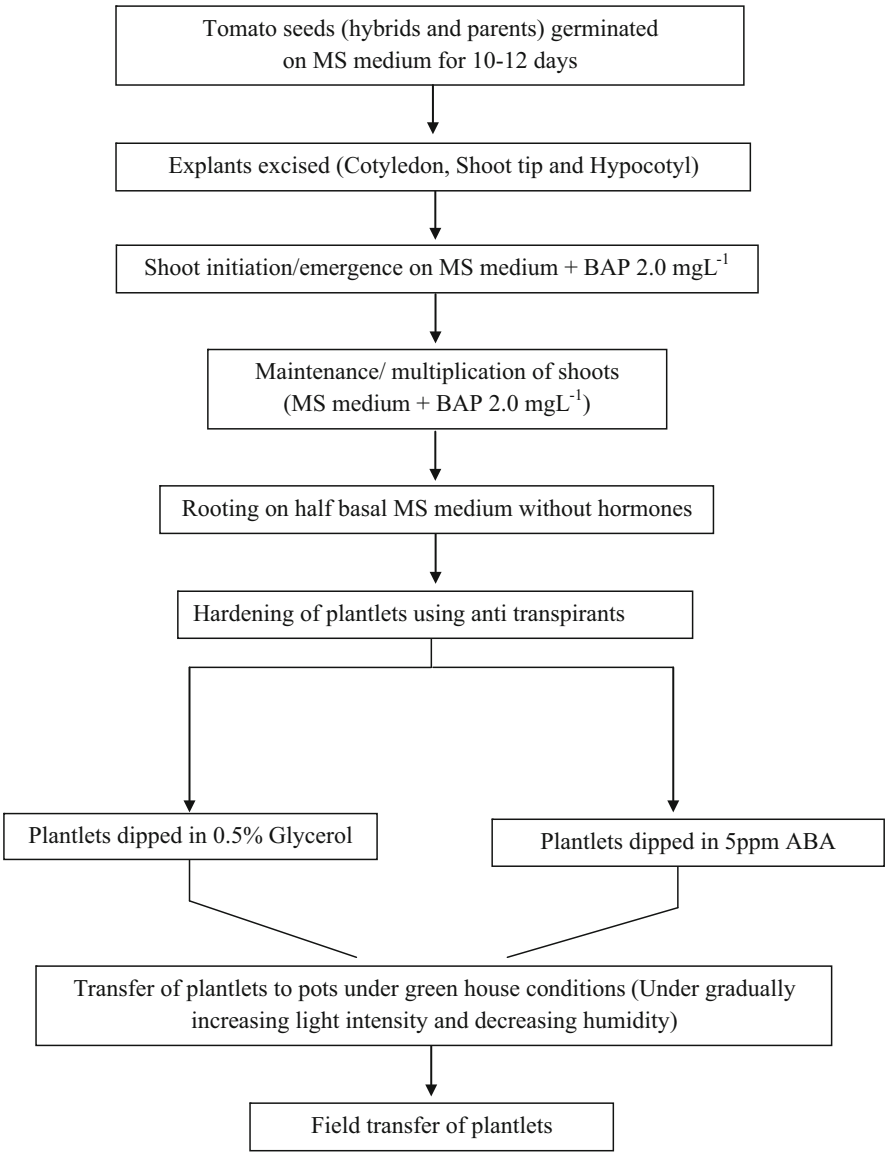


Fig. 16.1 Schematic diagram of protocol for micropropagation and hardening using antitranspirants in hybrids

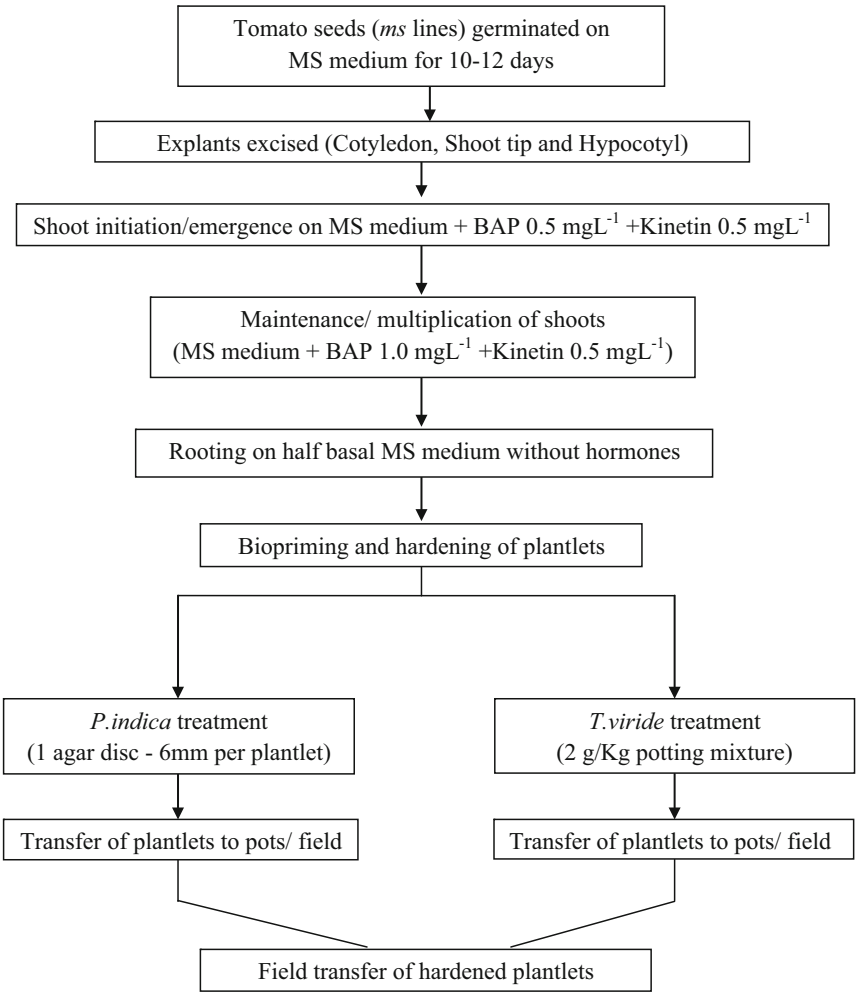


Fig. 16.2 Schematic diagram of protocol for micropropagation and biological hardening for *ms* lines

16.4.2 Establishment of Aseptic Cultures and Shoot Initiation

Five génotypes (two hybrids and three parents) gave maximum shoot induction/émergence response on MS medium containing 2.0 mg^{-1} BAP. The Accession-2 gave maximum shoot induction response (100%) on MS medium with 2.5 mg^{-1} BAP after 4 weeks of transfer. The shoot induction/emergence response revealed significant genotypic differences when cotyledons were cultured on medium with different concentrations of BAP (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mgl^{-1}) in these genotypes.

The optimum response towards shoot initiation/emergence in *ms* lines was observed from the cotyledons of all the three *ms* lines (EC251735, EC251692 and EC251752) on MS medium with BAP (0.5 mg/l) + Kinetin (0.5 mg/l). The genotypic differences were also observed in *ms* lines with relatively better response recorded in EC251692. In present studies, the success has been found to depend largely on the genotype, explant source and choice of plant growth regulators used. The genotypic differences for the requirement of specific level of cytokinins individually or in combination were also observed (Bhushan and Gupta 2010; El-Farash et al. 1993; Gupta and Khere 2002; Newman et al. 1996). Plant regeneration has also been reported using different explants like cotyledons, shoot tips (Newman et al. 1996; Narayanswamy and Ramaswamy 1995; Soniya et al. 2001; Venkatachalam et al. 2000).

16.4.3 Maintenance/Multiplication of Shoots and Rooting

The shoot buds or small shoots initiated on cotyledon as explants on MS medium containing 2 mgl^{-1} BAP were sub-cultured on the same medium with different concentrations of BAP for shoot elongation and multiple shoot formation. Best response was again observed on MS medium with 2 mgl^{-1}BAP. The maximum number of shoots per explant was observed in hybrid TH802 (5.17) when compared with its parents, Accession-2 (5.05) and Haelani (4.22). Similarly, hybrid TH2312 also gave maximum number of shoots per explant (5.11) when compared to its parents VFN8 (5.01) and Punjab Chuhara (4.94). The separated shoots were sub-cultured on MS medium supplemented with different concentrations and combinations of BAP and Kinetin and the best response was observed on the same medium supplemented with BAP (1 mg/l) + Kinetin (0.5 mg/l). Almost all the five *ms* lines responded towards establishment of cultures and their further multiplication. However, the line EC251692 exhibited relatively better response towards multiple shoot formation with 5.3 numbers of shoots per culture.

The separated shoots (4–5 cm) of hybrids and *ms* lines, when transferred to basal or half basal MS medium (without hormones), resulted in profuse rooting. However, genotypic differences were observed with respect to rooting. Among hybrids and parents, maximum number of roots per shoot and root length was recorded in hybrids (TH802 and TH2312). The plantlets have been produced in thousands in various cycles through reproducible and dependable micropropagation protocol developed in our laboratory.

16.4.4 Hardening and Field Transfer

In tomato hybrids and parental genotypes, plantlets after antitranspirant (Glycerol and ABA) treatments were transferred in small plastic pots containing pot mixture.

Glycerol (0.5%) and ABA (5 ppm) gave the best response after 3 weeks in hybrids with 71% and 58% survival, respectively. In *ms* lines, glycerol (0.7%) and ABA (5 ppm) proved best after 3 weeks with 68% and 66% survival, respectively. The better survival of acclimatized or primed tomato plantlets may be due to higher relative water content resulting from lower initial stomatal conductance and/or reduced transpiration losses.

Biological hardening data with different treatment combinations of *P. indica, T. viride* and antitranspirants in micropropagated plantlets of male sterile lines indicated usefulness of bioagents. The treatment with *P. indica* gave maximum survival (86.6%) followed by *T. viride* (75.0%) with minimum survival (36.6%) recorded in untreated control. The data recorded on plant height, number of branches, number of leaves, leaf length and leaf breadth has shown relatively better results in treatment with *T. viride* and comparable with *P. indica*. However, *P. indica* and *T. viride* when used in combination, showed synergetic effects for survival and growth under field conditions. The better survival of micropropagated plants on treatments with *P. indica* could be attributed to its growth promoting or antifungal property (Varma et al. 1999, 2001; Singh et al. 2003). *T. viride* when used alone or in combination with glycerol was also effective in improving survival, growth and vigor. The better compatibility of *T. viride* with antitranspirants over *P. indica* with antitranspirants could be attributed to relatively poor colonization of the latter with the roots of micropropagated plantlets or relatively better role of *T. viride* in control of soil borne pathogens or both. The role of *Trichoderma* in control of soil borne pathogens in plant kingdom has been reported by various workers (Sivan and Chet 1986).

The in vitro raised and hardened plantlets in hybrids/parents/*ms* lines were successfully transferred to field and expressed true-to-type characters in hybrids/parents. Male sterility was also expressed at maturity in micropropagated *ms* lines transferred to pots in the hardening facility or field. Acclimatized plants, though showed relatively better survival over direct transfer, yet the maximum survival did not exceed 41.66%.

16.5 Conclusion

The micropropagation and hardening protocol developed by our group for hybrids and male sterile lines in tomato (otherwise difficult to propagate through conventional means) using antitranspirants and bioagents is both reproducible and dependable and can be scaled up with minor modifications for commercial applications for specific genotype (hybrid or *ms* line or transgenic).

Acknowledgements The work on Micropropagation and hardening has been funded by Department of Biotechnology (GOI), New Delhi, India and NATP-ICAR (GOI), New Delhi, India. Authors are grateful to PAU, Ludhiana, India and NBPGR, New Delhi, India for supply of seeds. Authors also acknowledge thanks to Prof Ajit Varma, JNU, New Delhi, India and Mr RK Khajuria, IIM (CSIR), Jammu, India for making available *P. indica* and *T. viride* cultures, respectively.

References

Bai Y, Lindhout P (2007) Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? Ann Bot 100:1085–1094

Bhushan A, Gupta RK (2010) Adventitious shoot regeneration in different explants of six genotypes of tomato. Indian J Hortic 67:224–227

El-Farash EMN, Abdalla HI, Taghjian AS, Ahmad MH (1993) Genotype explant age and explant type as affecting callus and shoot regeneration in tomato. Assuit J Agr Sci 24:5–14

Gupta RK, Khere PK (2002) Micropropagation studies in male sterile line(s) in tomato. In: Biotechnology for sustainable hill agriculture, DARL, Pithoragarh, India, pp 109–111

Murashige T, Skoog F (1962) A revised medium for rapid growth and biossays with tobacco tissue cultures. Physiol Plant 15:473–497

Narayanswamy TC, Ramaswamy NM (1995) *In vitro* culture of tomato hybrids (*Lycopersicon esculentum* Mill.) In Vitro 31:77–79

Newman PO, Krishnaraj S, Saxena PK (1996) Regeneration of tomato (*Lycopersicon esculentum* Mill.), somatic embryogenesis and shoot organogenesis from hypocotyl explants induced with 6-benzylademine. Int J Plant Sci 157:554–560

Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, Peskan T, Hehl S, 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, 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

Sahay NS, Varma A (1999) *Piriformospora indica*: a new biological hardening tool for micropropagated plants. FEMS Microbiol Lett 181:297–306

Sahay NS, Varma A (2001) Biological approach towards increasing the survival rates of micropropagated plants. Curr Sci 78:126–129

Selvapandiyan A, Subramani J, Bhatt PN, Mehta AR (1988) A simple method for direct transplantation of cultured plants to the field. Plant Sci 56:81–83

Singh A, Singh A, Kumari M, Rai MK, Varma A (2003) Biological importance of *Piriformospora indica* – a novel symbiotic mycorrhiza like fungus: an overview. Indian J Biotechnol 2:65–75

Sivan A, Chet I (1986) Possible mechanisms for control of *Fusarium* spp. by *Trichoderma harzianum*. Pests and Diseases 2:865–872

Soniya EV, Banerjee NS, Das MR (2001) Genetic analysis of somaclonal variation among callus derived plants of tomato. Curr Sci 80:1213–1215

The Tomato Genome Consortium (2012) The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635–641

Varma A, Verma S, Sudha N, Sahay N, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivatble plant growth promoting root endophyte. Appl Environ Microbiol 65:2741–2744

Varma A, Singh A, Sudha N, Sharma J, Kumari M, Kranner I (2001) *Piriformospora indica*: a cultivable mycorrhiza – like endosymbiotic fungus. In: Hock B (ed) Mycota IX series. Springer, Berlin, pp 123–150

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 (2012) The symbiotic fungus *Piriformospora indica*: update. In: Hock B (ed) The mycota IX. Springer, Berlin, pp 21–254

Venkatachalam P, Geetha N, Priya P, Rajaseger G, Jayabalan N (2000) High frequency plantlet regeneration from hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.) via organogenesis. Plant Cell Biotechnol Mol Biol 1:95–100

Chapter 17
Piriformospora indica (*Serendipita indica*) Enhances Growth and Secondary Metabolites in *Cucurma longa*

Diksha Bhola, Ruchika Bajaj, Swati Tripathi, and Ajit Varma

Abstract Since Vedic times, haldi has been used as an important spice and in medicinal remedies. Haldi as commonly known in North India is the powdered form of dried rhizomes obtained from *Cucurma longa* L. The active ingredients of the plant are curcuminoids and essential oils. The curcuminoids and essential oils obtained from the plant have therapeutic properties against a diverse range of ailments. Further, the alkaloids are used in dermatology, cosmetology as well as perfumery. These properties render this plant of high economic and medicinal value. Thus increased production and yield of the plant is highly required. The root endophytic fungus *Piriformospora indica* (*Serendipita indica*) is exploited for enhanced production. The fungus increases growth and yield of the plant along with augmenting the secondary metabolities.

17.1 Introduction

Traditionally in different ethnic societies plants harbouring medicinal attributes have been used for treatment for generations now. However, scientific evidence in terms of modern medicine is lacking in most cases. One such widely cultivated Asian herb is *Cucurma longa* L. The powdered form called turmeric is used in traditional Indian medicines against cough, diabetic wounds, biliary disorders, hepatic disorders, anorexia, coryza, rheumatism, swelling and sinusitis. The powder is used in India since vedic culture nearly 4000 years ago. As stated in the Sanskrit,

D. Bhola • S. Tripathi • A. Varma (✉)
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3, Fourth Floor, Sector-125, Noida 201313, India
e-mail: ajitvarma@amity.edu

R. Bajaj
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, E-3, Fourth Floor, Sector-125, Noida 201313, India

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

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_17

Ayurveda and Unani systems turmeric powder has long been used as a medicine in Southern Asia (Govindarajan 1980; Remadevi et al. 2007). In northern parts of India it is commonly known as 'haldi' which is derived from Sanskrit word 'haridra' while in south it is locally known as 'Manjal'. Turmeric has 53 different names in Sanskrit based on its characteristics described (Prasad and Aggarwal 2011).

India is largest producer and exporter of turmeric followed by Bangladesh, Indonesia, China, South Asia and Caribbean islands (Norman 1991). In India, Tamil Nadu has the largest producing land and trading centre for turmeric. Thus rightfully referred to as 'Yellow city' or 'Turmeric city' (Prasad and Aggarwal 2011). The characteristic yellow colour is bestowed to turmeric by curcumin which is a polyphenolic antioxidant. Curcumin along with volatile oils are responsible to exhibit a variety of pharmacological effects including anti-inflammatory, anti-tumour, anti-HIV and anti-infectious properties. Traditionally rhizomes are boiled for approximately 40–60 min in alkaline conditions and later on sun dried for 10–15 days reducing moisture to 10–11%. These dried rhizomes are further processed into powdered form.

17.2 Morphology and Taxonomy

Turmeric is the powdered product obtained from rhizomes of the plant *Curcuma long* L. The perennial herbaceous plant belongs to the Zingiberaceae family (Priyadarsini 2014). There are approximately 133 species of curcuma worldwide which are used as a spice and for medicinal applications (Sasikumar 2005). Since it is a herbaceous plant, the plant reaches up to the height of 1m with alternately arranged leaves in two rows. From the leaves emerges a false stem. This false stem bears a terminal inflorescence with bright yellow flowers. The rhizomes produced by the plant are highly branched, cylindrical, aromatic and yellow to orange in colour. The rhizomes are used for the propagation of plant and commercial production of turmeric powder and other compounds.

17.3 Active Ingredients of Turmeric

The chemical composition of turmeric is diverse. Cultivation conditions, varieties and location drastically affect the quality and quantity of the different chemical compounds present in turmeric. Altogether 235 primary phenolic compounds and terpenoids have been distinguished from this plant (Li et al. 2011). The major bioactive ingredients of turmeric belong to the diarylheptanoids and are known as curcuminoids. Further the most common curcuminoid present in turmeric is curcumin (Fig. 17.1). However, the commercial curcumin is typically a mixture of the following three curcuminoids—71.5% curcumin, 19.4% demethoxycurcumin

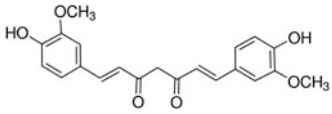


Fig. 17.1 Structure of Curcumin

and 9.1% of bisdemethoxycurcumin (Pfeiffer et al. 2003). Curcumin ($C_{21}H_{20}O_5$) was first isolated in 1815 and is known as diferuloyi methane or 1,6-heptadiene-3,5-diane-1,7bis(4-hydroxy-3-methoxyone-nyl) (Chempakam and Parthasarathy 2008). The compound has a molecular weight of 368.37, insoluble in water while solubilises in ethanol, acetone and methanol. Also three types of diarylpentanoids having a five carbon chain between two phenyl groups have been isolated from turmeric. The essential oils extracted from leaves as well as flowers are majorly constituted of monoterpenes. The commonly present terpenes are *p*-cymene, β-phellenderene, terpinolene, *p*-cymene-8-ol, cineole and myrcene. The aromatic smell and taste of dreied rhizomes are attributed to sesquiterpenes present in essential oils. The major identified sesquiterpenes are α-turmerone, β-turmerone, turmeronol A and turmeronol B.

17.4 Medicinal Applications

Curcumin is an immensely pleiotropic compound with the ability to stimulate the activity of various molecules involved in signalling (Gupta et al. 2011). Curcumin exhibits curative effects against a number of human ailments (Table 17.1).

17.5 Interactions of *Curcuma longa* L. with *Piriformospora indica* (*Serendipita indica*)

Piriformospora indica (*Serendipita indica*) is a mycorrhiza like axenically cultivable plant growth-promoting root endophyte (Weiß et al. 2016). It expansively colonizes the root hair zones inter- and intracellularly while excluding the elongation and meristematic zones (Deshmukh et al. 2006). Symbiotic relationship of *P. indica* with various taxonomically unrelated hosts increases plant growth and biomass (Peškan-Berghöfer et al. 2004; Waller et al. 2005; Shahollari et al. 2007; Sherameti et al. 2008; Camehl et al. 2010, 2011; Hilbert et al. 2012; Nongbri et al. 2012; Lahrmann et al. 2013; Varma et al. 2013; Prasad et al. 2013; Gill et al. 2016; Venus and Oelmuller 2013),

Table 17.1 Medicinal applications of turmeric

Property	Effect	Reference
Anti-microbial	Inhibited the bacterial growth Retarded the microbial growth, delayed the chemical changes, and extended the shelf life of rainbow trout Exhibited activity against histamine-producing bacteria Exhibited activity against food-borne pathogens Exhibited bactericidal activity Suppressed HBV replication in liver cells by enhancing the level of p53 protein Exhibited activity against *Trichophyton longifusus* Inhibited *Aspergillus flavus* growth and aflatoxin production	Mahady et al. (2002) Pezeshk et al. (2011) Paramasivam et al. (2007) Tayel and El-Tras (2009), Yano et al. (2006) Sathishkumar et al. (2010) Kim et al. (2009) Khattak et al. (2005) Sindhu et al. (2011) Wuthi-udomlert et al. (2000)
Insecticidal and larvicidal	Exhibited activity against *Sitophilus zeamais* and *Tribolium castaneum* Exhibited activity against the dengue vector *Aedes aegypti* Exhibited activity against *Anopheles stephensi* and *Culex quinquefasciatus* mosquito larvae Exhibited toxicity against red spider mites	Suthisut et al. (2011) Kalaivani et al. (2012) Singha and Chandra (2011) Svinningen et al. (2010)
Anti-oxidant	Ethanol extracts exhibited high anti-oxidant activities compared with aqueous extracts Protected renal cells against oxidative stress induced by H_2O_2 Exhibited anti-oxidant activity in in vitro assays Suppressed the incidence of atherosclerosis, exhibited hypolipidemic and anti-oxidant activities	Qader et al. (2011) Cohly et al. (1998) Betancor-Fernandez et al. (2003), Kurien and Scofield (2007) Jin et al. (2011)
Anti-mutagenic	Exhibited anti-mutagenicity against chemical-induced mutagenesis in bacteria Exhibited anti-mutagenicity to sodium azide, NPD, 2-AAF, and benzo[a]pyrene *in vitro* Reduced the formation of heterocyclic amines *in vitro* Exhibited anti-mutagenicity against IQ and 4-NQO mutagens	Azuine et al. (1992) Kuttan et al. (2004) Puangsombat et al. (2011) Peng et al. (2010)
Radioprotection	Protected against gamma-radiation–induced inactivation of bacterial strains Protected against X-ray–induced DNA damage	Sharma et al. (2000) Pal and Pal (2005)
Other activities	Exhibited chemoprotective activity against benzo[a]pyrene-induced chromosomal damage Recovered the cells from cisplatin-induced nephrotoxicity	Ghaisas and Bhide (1994) Sohn et al. (2009) Saelee et al. (2011) Mohankumar and McFarlane (2011)

(continued)

Table 17.1 (continued)

Property	Effect	Reference
	Exhibited anti-psoriatic activity and down-regulated the expression of CSF-1, IL-8, NF-B1, and NF-B2 Stimulated insulin secretion under basal and hyperglycemic conditions Inhibited human pancreatic amylase activity Exhibited immuno-stimulatory activity in human PBMCs Inhibited A fibril aggregation in a cell-free assay	Ponnusamy et al. (2011) Yue et al. (2010) Shytle et al. (2009)
Anti-inflammation	Reduced the inflammation in patients with chronic localized or generalized periodontitis	Behal et al. (2011)
Lupus nephritis	Decreased proteinuria, hematuria, and systolic blood pressure in patients with relapsing or refractory lupus nephritis	Khajehdehi et al. (2012)
Anticancer	Significantly reduced NO level in CML patients when given alone or in combination with imatinib Produced remarkable symptomatic relief in patients with external cancerous lesions Reduced the ulcer size in patients with peptic ulcer	Ghalaut et al. (2012) Kuttan et al. (1987) Prucksunand et al. (2001)
Antidiabetic	Attenuated proteinuria, TGF, and IL-8 in patients with overt type 2 diabetic nephropathy Increased postprandial serum insulin levels, insignificant effect on plasma glucose levels and the glycemic index	Khajehdehi et al. (2011) Wickenberg et al. (2010)
Irritable bowel syndrome	Improved the symptoms of IBS and reduced the prevalence of disease Increased the bowel motility and activated the hydrogen-producing bacterial flora in the colon	Bundy et al. (2004) Shimouchi et al. (2009)
Antimutagenic	Significantly reduced the urinary excretion of mutagens in smokers	Polasa et al. (1992)
Protection from fibrosis	Offered protection against benzo[a]pyrene-induced increases in micronuclei in circulating lymphocytes of healthy subjects; decreased the number of micronucleated cells in patients with submucous fibrosis	Hastak et al. (1997)

higher seed yield, early flowering, and biotic and abiotic stress tolerance responses (Baltruschat et al. 2008; Singh et al. 2011; Bajaj et al. 2015; Gill et al. 2016). It has been reported that mutualistic associations of this fungus stimulates increased allocation of nutrients like phosphate to the plant roots (Yadav et al. 2010).

Exploiting these plant benefitting properties, a formulation of *P. indica* with magnesium sulphite was prepared where magnesium sulphite acts as a carrier. For this, 2% (w/w) of fungal biomass served as effective and stable formulation. On an

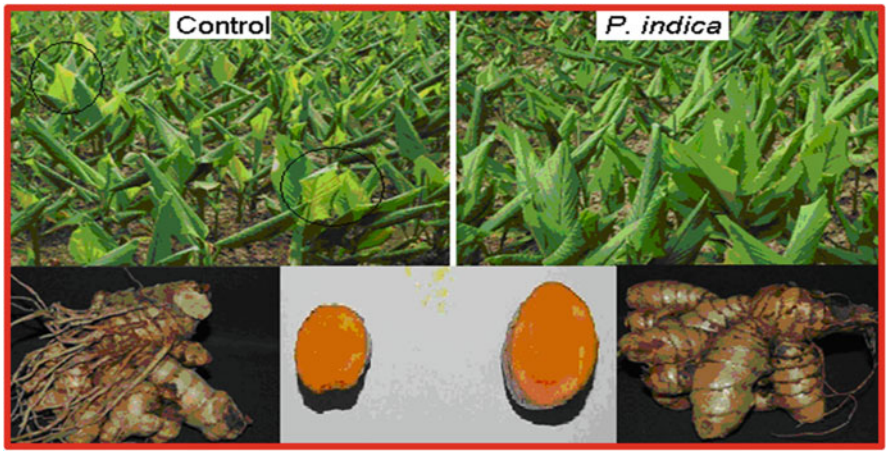


Fig. 17.2 Interaction studies of *P. indica* with *Curcuma longa*

Table 17.2 Increase percentage of active compounds

Parameters	Percent increase over control
Volatile oil	21.09
Curcumin	19.00

Table 17.3 Difference in yield of treated versus control plants

Yield in quintal/kanal	
Control	Treated
13q	14.65q

average the colony forming unit (CFU) count was maintained as 10^9 and moisture content was 20%.

To study the effects of endophytic fungus, *P. indica*, rhizomes of turmeric were thoroughly mixed with the respective formulation and transferred to field. The field trials showed a beneficial association of *Curcuma longa* with *P. indica* (Bajaj et al. 2014). There is a distinctive increase in leaf number, roots and overall yield of *P. indica* treated plants produced rhizomes of bigger size as compared to the rhizomes in control (Fig. 17.2). An overall 12.69, 21.09 and 19.00%, respectively, increase was observed in yield, volatile oil and curcumin content respectively (Tables 17.2 and 17.3).

17.6 Mechanism for Enhanced Growth

Both the endophytic fungi and the host recognize each other during the early stages of colonization. Immediately after colonization there occurs an elevation in intracellular calcium levels in plant cells. This acts as an initial signalling stage in

interaction of mycorrhiza with plants (McAinsh and Pittman 2009). However, in various signalling pathways Ca^{2+} ions are the secondary messengers that transfer extracellular stimuli to the intracellular machinery and produce plant response (Sanders et al. 2002). Interaction of mycorrhizal fungi with host results in enhanced plant performance through elevation in nuclear as well as cytoplasmic Ca^{2+} levels (Vadassery and Oelmüller 2009). *In vitro* studies have shown that *P. indica* induces tuber along with yield in potato attributing to the increased expression of two Ca^{2+} dependent proteins such as CaM1, St-CDPK1 and lipoxygenase (Lox) mRNA (Upadhyaya et al. 2013). Various signals are exchanged at the surface where *P. indica* interacts with the plant. This results in an efflux of calcium and influx of phosphorous within plant cells (Yadav et al. 2010; Ansari et al. 2013). These external stimuli indicate involvement of phytohormones in programmed cell death (PCD) *via* endoplsmic stress or be directly involved in growth and development of the host plant (Qiang et al. 2011; Ansari et al. 2013). Mutants impaired in GA and JA exhibited low colonization, plant growth and development (Schäfer and Kogel 2009; Jacobs et al. 2011). Generally the calcium ions and phytohormones acting as signals down regulate the PRIb, PR5, β-gulcanase and upregulates auxin genes (Hückelhoven 2004; Lam 2004; Deshmukh and Kogel 2007; Trivedi et al. 2013). Further, the elevated growth of plants associated with *P. indica* is linked with an increased uptake of nutrients such as phosphorous and nitrogen (Oelmüller et al. 2009; Sherameti et al. 2005). It has been studied that *P. indica* stimulates increased transcription of nitrate reductase gene and helps in nitrate uptake from soil. Also, *P. indica* facilitates the transport of phosphate ions through specialized phosphate transporters (Yadav et al. 2010). It also facilitates the uptake of macro-nutrients such as Iron, Zinc, Manganese and Copper from the soil (Oelmüller et al. 2009) (Fig. 17.3).

17.7 Plausible Mechanism of Enhanced Curcumin Levels

As proposed by Kita et al. (2008) the biosynthetic pathways for synthesis of curcuminoids initiates from phenylalanine (Fig. 17.4). It includes two units of cinnamic acid and one central carbon from malonic acid. It is further carried out by addition of functional groups on aromatic rings. Curcuminoid synthase (CUS) catalyses the formation of curcuminoids *via* phenylpropanoid CoAs and malonyl CoA through 3-oxo-5-phenyl-pent-4-enoic acid. Since curcuminoids consist of p-coumaroyl and feroloyl moieties as a part of their structure. Thus it is proposed that cinnamoyl CoA is also a substrate in curcuminoid biosynthesis.

As indicated earlier the colonization of *P. indica* initiates a cascade of Ca^{2+} and phytohormone signalling in the host plants. Calcium ions and phytohormone signalling in the host plants. Calcium ions and phytohormones such as Auxin, GA and Abscisic acid stimulate expression of various genes involved in plant growth. It is proposed that the increased transcription of genes increases concentration of substrates involved in curcumin biosynthesis. This in turn increases

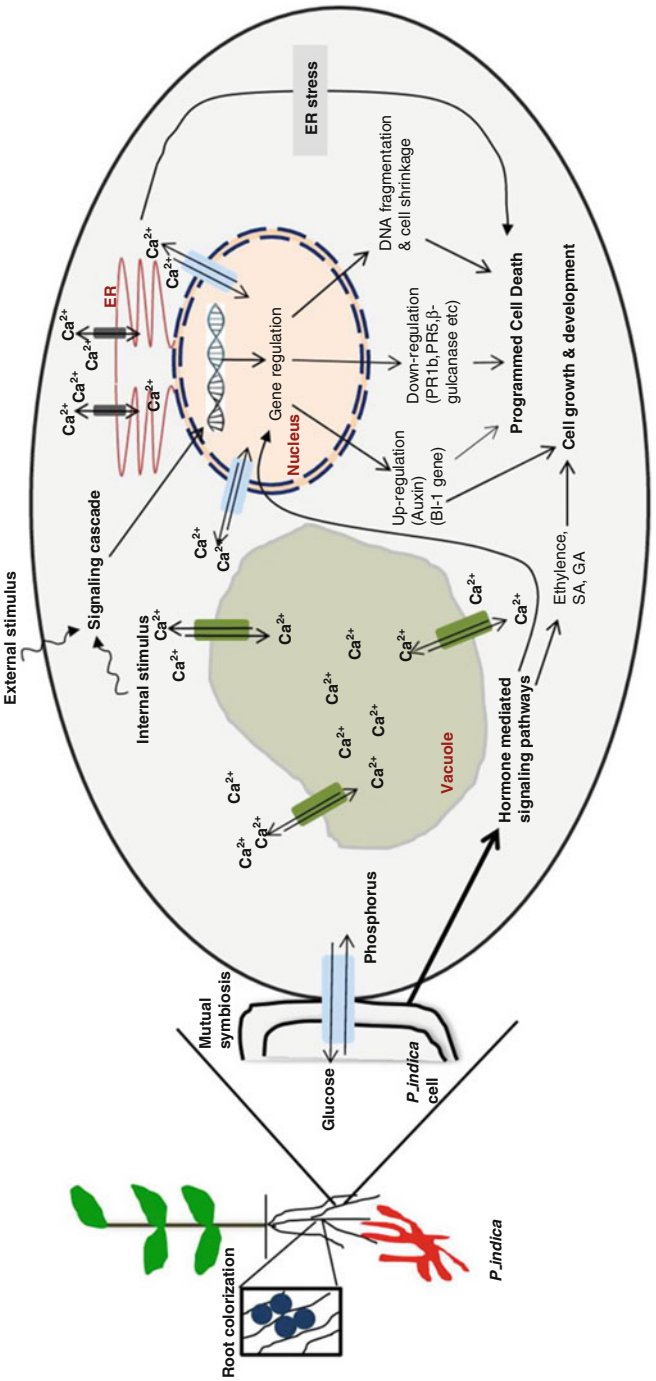


Fig 17.3 Schematic representation of cellular and biochemical signalling cascades mediated by *Piriformospora indica* and plant colonization signalling (Hückelhoven 2004; Lam 2004; Deshmukh et al. 2006; Deshmukh and Kogel 2007; Schäfer and Kogel 2009; Vadassery and Oelmüller 2009; Jacobs et al. 2011, Qiang et al. 2011)

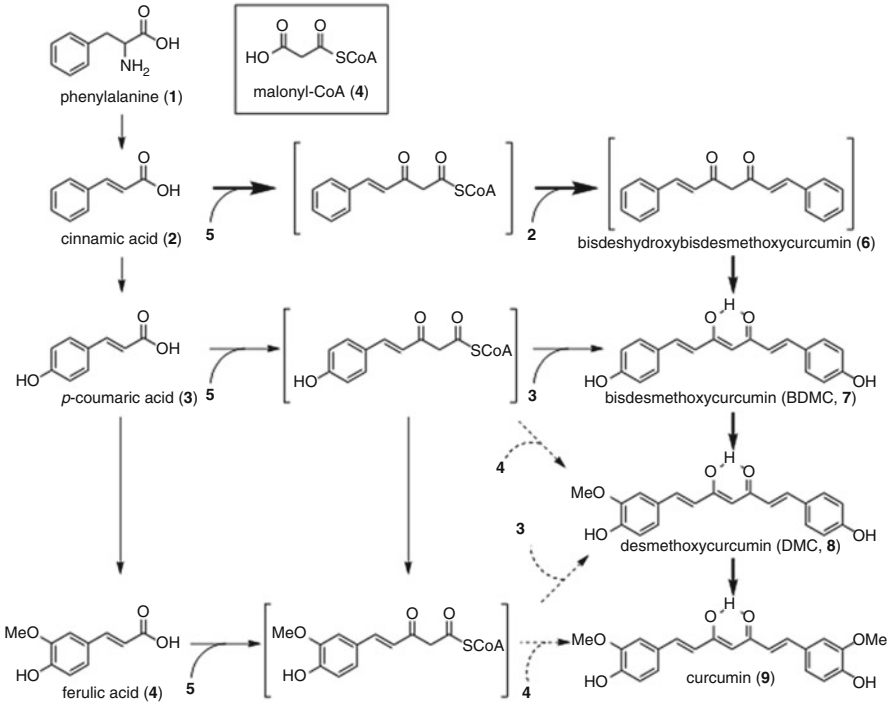


Fig. 17.4 Proposed major (*thick arrows*) and minor (*thin* and *dotted arrows*) pathways to Curcuminoids in turmeric. *1* Phenylalanine; *2* cinnamic acid; *3* p-coumaric acid; *4* ferulic acid; *5* malonic acid; *6* putative curcuminoid skeleton intermediate: bisdeshydroxybisdesmethoxycurcumin (BDHBDMC); *7* bisdesmethoxycurcumin (BDMC); *8* desmethoxycurcumin (DMC); *9* curcumin

concentration of curcumin produced. Also *P. indica* stimulates activities of various enzymes involved in plant biosynthesis which increases overall plant growth and secondary metabolism.

17.8 Future Aspects

The root endophytic mycorrhizal fungus, *P. indica* promotes growth and development of turmeric plants. The increased growth is attributed to enhanced allocation of nutrients from the soil to plants. The fungus also provides resistance to biotic and abiotic stresses encountered by the plants. It is well established that turmeric is a plant with imminent medicinal value. Thus application of *P. indica* for enhanced growth would be beneficial for optimised production of natural curcuminoids and essential oils. Further, for better understanding the mechanisms involved in increased production of curcumin various molecular studies are preferred. Analysis

of genes involved in curcumin biosynthesis and effect of *P. indica* on expression of these genes could be studied. Molecular and genetic analysis studies will thus aid in better understanding and exploitation of this extremely potent medical compound. This demarcates a way to modern therapeutic medicines for a number of ailments.

References

Ansari MW, Bains G, Shukla A, Pant RC, Tuteja N (2013) Low temperature stress ethylene and not Fusarium might be responsible for mangomal formation. Plant Physiol Biochem 69:34–38

Azuine MA, Kayal JJ, Bhide SV (1992) Protective role of aqueous turmeric extract against mutagenicity of direct-acting carcinogens as well as benzo [alpha] pyreneinduced genotoxicity and carcinogenicity. J Cancer Res Clin Oncol 118:447–452

Bajaj R, Agarwal A, Rajpal K, Asthana S, Prasad R, Kharkwal AC, Kumar R, Sherameti I, Oemüller R, Varma A (2014) Co-cultivation of *Curcuma longa* with *Piriformospora indica* enhances the yield and active ingredients. Am J Curr Microbiol 2:1–12

Bajaj R, Hu W, Huang YY, 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

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schafer 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

Behal R, Mali AM, Gilda SS, Paradkar AR (2011) Evaluation of local drug-delivery system containing 2% whole turmeric gel used as an adjunct to scaling and root planing in chronic periodontitis: a clinical and microbiological study. J Indian Soc Periodontol 15:35–38

Betancor-Fernandez A, Perez-Galvez A, Sies H, Stahl W (2003) Screening pharmaceutical preparations containing extracts of turmeric rhizome, artichoke leaf, devil's claw root and garlic or salmon oil for antioxidant capacity. J Pharm Pharmacol 55:981–986

Bundy R, Walker AF, Middleton RW, Booth J (2004) Turmeric extract may improve irritable bowel syndrome symptomology in otherwise healthy adults: a pilot study. J Altern Complement Med 10:1015–1018

Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J, Oelmuller R (2010) Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytol 185:1062–1073

Camehl I, Drzewiecki C, Vadassery J, Shahollari B, Sherameti I, Forzani C, Munnik T, Hirt H, Oelmuller R (2011) The OXI1 kinase pathway mediates *Piriformospora indica*-induced growth promotion in *Arabidopsis*. Plos Pathogen 7:e1002051

Chempakam B, Parthasarathy VA (2008) Turmeric. In: Parthasarathy VA, Chempakam B, Zachariah TJ (eds) Chemistry of spice. CABI, Cambridge, pp 97–123

Cohly HH, Taylor A, Angel MF, Salahudeen AK (1998) Effect of turmeric, turmerin and curcumin on H_2O_2-induced renal epithelial (LLC-PK1) cell injury. Free Radic Biol Med 24:49–54

Deshmukh SD, Kogel KH (2007) *Piriformospora indica* protects barley from root rot caused by *Fusarium graminearum*. J Plant Dis Protect 114:263–268

Deshmukh S, Hueckelhoven R, Schaefer 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 USA 103:18450–18457

Ghaisas SD, Bhide SV (1994) In vitro studies on chemoprotective effect of Purnark against benzo (a)pyrene-induced chromosomal damage in human lymphocytes. Cell Biol Int 18:21–27

Ghalaut VS, Sangwan L, Dahiya K, Ghalaut PS et al (2012) Effect of imatinib therapy with and without turmeric powder on nitric oxide levels in chronic myeloid leukemia. J Oncol Pharm Pract 18:186–190

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. https://doi.org/10.3389/fmicb.2016.00332

Govindarajan VS (1980) Turmeric-chemistry, technology, and quality. Crit Rev Food Sci Nutr 12:199–301

Gupta SC, Prasad S, Kim JH, Patchva S, Webb LJ et al (2011) Multi targeting by curcumin as revealed by molecular interaction studies. Nat Prod Rep 28:1937–1955

Hastak K, Lubri N, Jakhi SD, More C et al (1997) Effect of turmeric oil and turmeric oleoresin on cytogenetic damage in patients suffering from oral submucous fibrosis. Cancer Lett 116:265–269

Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A (2012) Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol 196:520–534

Hückelhoven R (2004) BAX Inhibitor-1, an ancient cell death suppressor in animals and plants with prokaryotic relatives. Apoptosis 9:299–307

Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V et al (2011) Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. Plant Physiol 156:726–740

Jin S, Hong JH, Jung SH, Cho KH (2011) Turmeric and laurel aqueous extracts exhibit in vitro anti-atherosclerotic activity and in vivo hypolipidemic effects in a zebrafish model. J Med Food 14:247–256

Kalaivani K, Senthil-Nathan S, Murugesan AG (2012) Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae). Parasitol Res 110:1261–1268

Khajehdehi P, Pakfetrat M, Javidnia K, Azad F et al (2011) Oral supplementation of turmeric attenuates proteinuria, transforming growth factor-beta and interleukin-8 levels in patients with overt type 2 diabetic nephropathy: a randomized, double-blind and placebo-controlled study. Scand J Urol Nephrol 45:365–370

Khajehdehi P, Zanjaninejad B, Aflaki E, Nazarinia M et al (2012) Oral supplementation of turmeric decreases proteinuria, hematuria, and systolic blood pressure in patients suffering from relapsing or refractory lupus nephritis: a randomized and placebo-controlled study. J Ren Nutr 22:50–57

Khattak S, Saeed ur R, Ullah Shah H, Ahmad W et al (2005) Biological effects of indigenous medicinal plants *Curcuma longa* and *Alpinia galanga*. Fitoterapia 76:254–257

Kim HJ, Yoo HS, Kim JC, Park CS et al (2009) Antiviral effect of *Curcuma longa* Linn extract against hepatitis B virus replication. J Ethnopharmacol 124:189–196

Kita T, Imai S, Sawasa H, Kumagai H, Seto H (2008) The biosynthetic pathway in tumeric (*Curcuma longa*) as revealed by ^{13}C-labelled precursors. Biosci Biotechnol Biochem 72:1789–1798

Kurien BT, Scofield RH (2007) Curcumin/turmeric solubilized in sodium hydroxide inhibits HNE protein modification—an in vitro study. J Ethnopharmacol 110:368–373

Kuttan R, Sudheeran PC, Josph CD (1987) Turmeric and curcumin as topical agents in cancer therapy. Tumor 73:29–31

Kuttan R, Kuttan G, Joseph S, Ajith TA et al (2004) Antimutagenicity of herbal detoxification formula Smoke Shield against environmental mutagens. J Exp Clin Cancer Res 23:61–68

Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR et al (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. Proc Natl Acad Sci USA 110:13965–13970

Lam E (2004) Controlled cell death, plant survival and development. Nat Rev Mol Cell Biol 5:305–315. https://doi.org/10.1038/nrm1358

Li S, Yuan W, Deng G, Wang P, Yang P et al (2011) Chemical composition and product quality control of turmeric (*Curcuma longa* L.). Pharm Crops 2:28–54

Mahady GB, Pendland SL, Yun G, Lu ZZ (2002) Turmeric (*Curcuma longa*) and curcumin inhibit the growth of *Helicobacter pylori*, a group 1 carcinogen. Anticancer Res 22:4179–4181

McAinsh MR, Pittman JK (2009) Shaping the calcium signature. New Phytol 181:275–294

Mohankumar S, McFarlane JR (2011) An aqueous extract of *Curcuma longa* (turmeric) rhizomes stimulates insulin release and mimics insulin action on tissues involved in glucose homeostasis in vitro. Phytother Res 25:396–401

Nongbri PL, Johnson JM, Sherameti I, Glawischnig E, Halkier BA, Oelmüller R (2012) Indole-3-acetaldoxime-derived compounds restrict root colonization in the beneficial interaction between *Arabidopsis* roots and the endophyte *Piriformospora indica*. Mol Plant Microbe Interact 25:1186–1197

Norman J (1991) The complete book of spices. Viking Studio Books, Penguin Books USA Inc, New York, NY

Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica,* a cultivable root endophyte with multiple biotechnological applications. Symbiosis 19:1–19

Pal A, Pal AK (2005) Radioprotection of turmeric extracts in bacterial system. Acta Biol Hung 56:333–343

Paramasivam S, Thangaradjou T, Kannan L (2007) Effect of natural preservatives on the growth of histamine producing bacteria. J Environ Biol 28:271–274

Peng CH, Chiu WT, Juan CW, Mau JL et al (2010) Pivotal role of curcuminoids on the antimutagenic activity of *Curcuma zedoaria* extracts. Drug Chem Toxicol 33:64–76

Peškan-Berghöfer T, Shahollari B, Giang PH, Hehl S, Markert C, Blanke V, Varma AK, Oelmüller R (2004) Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. Physiol Plant 122:465–477

Pezeshk S, Rezaei M, Hosseini H (2011) Effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4 ± 1C. J Food Sci 76:387–391

Pfeiffer E, Hhle S, Solyom A, Metzler M (2003) Studies on the stability of turmeric constituents. J Food Eng 56:257–259

Polasa K, Raghuram TC, Krishna TP, Krishnaswamy K (1992) Effect of turmeric on urinary mutagens in smokers. Mutagenesis 7:107–109

Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S et al (2011) Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evid Based Complement Alternat Med 2011:1–10

Prasad S, Aggarwal BB (2011) Turmeric, the golden spice: from traditional medicine to modern medicine. Herbal medicine: biomolecular and clinical aspects, oxidative stress and disease series. CRC Press, USA, pp 259–284

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

Priyadarsini KI (2014) The chemistry of curcumin: from extraction to therapeutic agent. Molecules 19:20091–20112

Prucksunand C, Indrasukhsri B, Leethochawalit M, Hungspreugs K (2001) Phase II clinical trial on effect of the long turmeric (*Curcuma longa* Linn) on healing of peptic ulcer. Southeast Asian J Trop Med Public Health 32:208–215

Puangsombat K, Jirapakkul W, Smith JS (2011) Inhibitory activity of Asian spices on heterocyclic amines formation in cooked beef patties. J Food Sci 76:174–180

Qader SW, Abdulla MA, Chua LS, Najim N et al (2011) Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. Molecules 16:3433–3443

Qiang X, Weiss M, Kogel KH, Schäfer P (2011) *Piriformospora indica*—a mutualistic basidiomycete with an exceptionally large plant host range. Mol Plant Pathol 13:508–518

Remadevi R, Surendran E, Kimura T (2007) Turmeric in traditional medicine. In: Ravindran PN, Nirmal Babu K, Sivaraman K (eds) Turmeric: the genus Curcuma. CRC Press, Boca Raton, London, New York, pp 409–436

Saelee C, Thongrakard V, Tencomnao T (2011) Effects of Thai medicinal herb extracts with antipsoriatic activity on the expression on NF-kappaB signaling biomarkers in HaCaT keratinocytes. Molecules 16:3908–3932

Sanders D, Pelloux J, Brownlee C, Harper JF (2002) Calcium at the crossroads of signalling. Plant Cell 14:401–417

Sasikumar B (2005) Genetic resources of *Curcuma*: diversity, characterization and utilization. Plant Gen Resour 3:230–251

Sathishkumar M, Sneha K, Yun YS (2010) Immobilization of silver nanoparticles synthesized using *Curcuma longa* tuber powder and extract on cotton cloth for bactericidal activity. Bioresour Technol 101:7958–7965

Schäfer P, Kogel KH (2009) The Sebacinoid fungus *Piriformospora indica*, an orchid mycorrhiza which may increase host plant reproduction and fitness. In: Deising H (ed) Plant relationships. Springer, Berlin, pp 99–112

Shahollari B, Vadassery J, Varma A, Oelmueller R (2007) A leucine-rich repeat protein is required for growth promotion and enhanced seed production mediated by the endophytic fungus *Piriformospora indica* in *Arabidopsis thaliana*. Plant J 50:1–13

Sharma A, Gautam S, Jadhav SS (2000) Spice extracts as dose-modifying factors in radiation inactivation of bacteria. J Agric Food Chem 48:1340–1344

Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmuller R (2005) The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. J Biol Chem 280:26241–26247. https://doi.org/10.1074/jbc.M500447200

Sherameti I, Tripathi S, Varma A, Oelmuller R (2008) The root-colonizing endophyte *Pirifomospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Mol Plant Microbe Interact 21:799–807. https://doi.org/10.1094/MPMI-21-6-0799

Shimouchi A, Nose K, Takaoka M, Hayashi H et al (2009) Effect of dietary turmeric on breath hydrogen. Dig Dis Sci 54:1725–1729

Shytle RD, Bickford PC, Rezai-zadeh K, Hou L et al (2009) Optimized turmeric extracts have potent antiamyloidogenic effects. Curr Alzheimer Res 6:564–571

Sindhu S, Chempakam B, Leela NK, Suseela Bhai R (2011) Chemoprevention by essential oil of turmeric leaves (*Curcuma longa* L.) on the growth of *Aspergillus flavus* and aflatoxin production. Food Chem Toxicol 49:1188–1192

Singh LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6:175–191

Singha S, Chandra G (2011) Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*. Asian Pac J Trop Med 4:288–293

Sohn SH, Lee H, Nam JY, Kim SH et al (2009) Screening of herbal medicines for the recovery of cisplatin-induced nephrotoxicity. Environ Toxicol Pharmacol 28:206–212

Suthisut D, Fields PG, Chandrapatya A (2011) Contact toxicity, feeding reduction, and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. J Econ Entomol 104:1445–1454

Svinningen AE, Rashani KP, Jegathambigai V, Karunaratne MD et al (2010) Efficacy of *Curcuma aeruginosa* rhizome and Adhatoda vasica plant extracts, on red spider mite, *Tetranychus urticae* in *Livistona rotundifolia*. Commun Agric Appl Biol Sci 75:391–397

Tayel AA, El-Tras WF (2009) Possibility of fighting food borne bacteria by Egyptian folk medicinal herbs and spices extracts. J Egypt Public Health Assoc 84:21–32

Trivedi DK, Bhatt H, Pal R, Johri AK, Tuteja N, Bhavesh NS (2013) Sequence specific 1H, 13C and 15N NMR assignments of cyclophilin A like protein from *P. indica* involved in salt tolerance. Biomol NMR Assign 7:175–178

Upadhyaya CP, Gururani MA, Prasad R, Varma A (2013) A cell wall extract from *Piriformospora indica* promotes tuberization in potato (*Solanum tuberosum* L.) via enhanced expression of CaC2 signaling pathway and lipoxygenase gene. Appl Biochem Biotechnol 170:743–755

Vadassery J, Oelmüller R (2009) Calcium signalling in pathogenic and beneficial plant microbe interactions: what can we learn from the interaction between *Piriformospora indica* and *Arabidopsis thaliana*. Plant Signal Behav 4:1024–1027. https://doi.org/10.4161/psb.4.11.9800

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

Venus Y, Oelmüller R (2013) Arabidopsis ROP1 and ROP6 influence germination time, root morphology, the formation of F-actin bundles, and symbiotic fungal interactions. Mol Plant 6:872–886

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, 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 USA 102:13386–13391. https://doi.org/10.1073/pnas.0504423102

Weiß M, Waller F, Zuccaro A, Selosse M-A (2016) Sebacinales-one thousand and one interactions with land plants. New Phytol 211:20–40

Wickenberg J, Ingemansson SL, Hlebowicz J (2010) Effects of *Curcuma longa* (turmeric) on postprandial plasma glucose and insulin in healthy subjects. Nutr J 9:43. https://doi.org/10.1186/1475-2891-9-43

Wuthi-udomlert M, Grisanapan W, Luanratana O, Caichompoo W (2000) Antifungal activity of *Curcuma longa* grown in Thailand. Southeast Asian J Trop Med Public Health 31:178–182

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

Yano Y, Satomi M, Oikawa H (2006) Antimicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. Int J Food Microbiol 111:6–11

Yue GG, Chan BC, Hon PM, Kennelly EJ et al (2010) Immunostimulatory activities of polysaccharide extract isolated from *Curcuma longa*. Int J Biol Macromol 47:342–347

Chapter 18
Effect of *Azotobacter chroococcum* and *Piriformospora indica* on *Oryza sativa* in Presence of Vermicompost

Sarita Manandhar, Reshma Tuladhar, Kamil Prajapati, Anjana Singh, and Ajit Varma

Abstract The overall growth and development of various plants is benefited by the activity of rhizospheric microorganisms in soil. The beneficial activities of these organisms include biological nitrogen fixation, phosphate solubilization and mineralization etc. which is enhanced in presence of mycorrhiza. A pot culture trial was carried out to study the growth promotion of *Oryza sativa* with dual inoculation of *Azotobacter chroococcum* and *Piriformospora indica* an Arbuscular-Mycorrhiza-like-fungus in presence of vermicompost. The study parameters included to evaluate the dual effect were shoot length, root length, fresh and dry shoot weight, fresh and dry root weight, panicle numbers, plant tissue analysis (NPK content) and soil analysis in terms of pH, NPK and organic content on 45th and 90th day for vegetative and reproductive stage respectively. In both stages, a significant positive response was observed in all growth parameters when plant was dual inoculated with *A. chroococcum* and *P. indica* in presence of vermicompost than untreated control plants. The result shows that the combination of dual inoculation of *A. chroococcum* and *P. indica* with vermicompost was found to improve growth parameters and nutrient uptake in rice plant.

S. Manandhar
Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal

R. Tuladhar • K. Prajapati • A. Singh (✉)
Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
e-mail: anjanas67@gmail.com

A. Varma
Amity Institute of Microbial Technology, Amity University Uttar Pradesh, Fourth Floor, E-3 Sector-125, Noida 201313, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_18

18.1 Introduction

Growth of plant requires fundamental nutrients like nitrogen, phosphorus, potassium etc. Nitrogen though present abundantly on earth surface in the form of nitrogen gas is unavailable to most living organisms. Inert nitrogen gas needs to be converted into organic form through biological nitrogen fixation to be available for organisms (Deacon 2006). Biological nitrogen fixation is usually associated with symbiotic *Rhizobium*-legume system for improving the fertility and productivity (Varma et al. 2013). Beside these, numerous free living soil bacteria of the genera *Azotobacter* (aerobic), *Clostridium* (strictly anaerobic), *Klebsiella* (facultative aerobic) and *Rhodospirillium* (anaerobic photosynthetically active) are capable of fixing significant atmospheric nitrogen in the absence of legumes (Vadakattu and Paterson 2006). The possibility of using *Azotobacter chroococcum* as microbial inoculant through production of growth substances and their effects on the plant has markedly enhanced crop production in agriculture. Free living N_2-fixer diazotroph of genus *Azotobacter* synthesizes auxins, cytokinins, and gibberellic acid like substances and these growth materials are the primary substances regulating the enhanced plant growth. It stimulates rhizospheric microbes, protects the plants from phyto-pathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation (Cruz et al. 2013).

The consortium of plant–microbe interaction is complex encompassing diverse species in soil. Mycorrhizal fungi and soil microorganisms influence the growth and nutrient uptake of plant. Microbial compounds produced by soil microorganisms increase root cell permeability which enhance symbiotic association of mycorrhizal fungi and plant. On the other hand root exudates modified as a consequence of mycorrhizal association affect soil microbial population (Saranya and Kumutha 2011).

Arbuscular Mycorrhiza (AM) fungi are the most common mycorrhiza associated with a vast taxonomic range of plants (Malla et al. 2002). The multitude benefits of arbuscular mycorrhizal association are improved nutrient uptake, mineralization of organic nutrients, resistance to abiotic and biotic stress, etc (Cruz et al. 2013). Being obligate, AM fungi thrives only on the living cells of the host plant thus is difficult to produce inoculums (Declerck et al. 2005).

Piriformospora indica, a non-obligate biotroph with ability to grow in synthetic medium has potential for agricultural application. This axenically cultivable arbuscular-mycorrhiza like fungus was named for its characteristic pear shaped spore morphology (Varma et al. 1999, 2012; Siddhanta et al. 2017). *P. indica*, a root colonizing and growth promoting basidiomycetes fungus is versatile with a broad spectrum of colonization to diverse plant species. It forms thin walled, haphazardly septate, hyaline and multinucleate hyphae and produces ovoid shaped chlamydospores to stabilize the interaction. In colonized crop plants, it confer various physiologically functional traits such as water and mineral uptake, photosynthesis, improved biomass, increased productivity and enhanced plant fitness to environmental stress (Prasad et al. 2013; Tuladhar et al. 2013; Ansari et al. 2014). The most

notable advantage of this mutualistic fungus is that it enhances the uptake of diffusion limited materials as phosphorus, copper, potassium, zinc and sulphur (Andrade-Linares et al. 2013).

Vermicompost is a mixture of worm manure, microbially decomposed matter and some partially decomposed organic matter that can be an option of chemical fertilizer to improve soil fertility. The nutrient content of vermicompost is higher than other compost and serves as an excellent soil additive for the improvement of soil quality (Prajapati et al. 2010). In order to guarantee the high effectiveness of inoculants and microbiological fertilizers it is necessary to find the compatible partners, i.e. a particular plant genotype and a particular *Azotobacter* strain that will form a good association with AM fungi (Prajapati et al. 2010). *P. indica* has been found to be a potent candidate symbiont for providing enormous growth promoting activity to a broad spectrum of plants including *Oryza sativa* in combination with rhizospheric microorganisms like *A. chroococcum* in presence of vermicompost.

18.2 Interaction of *P. indica* with Other Microorganisms

The interaction of mycorrhizal fungi with plants is affected in various ways by other microorganisms, especially by rhizospheric bacteria. Microbial communities in rhizosphere interact with each other and form a basis of a cumulative impact on plant growth. Plant development may be improved by the combination of mycorrhizal fungi and rhizosphere microorganisms acting in coordination at the root soil interface. In natural terrestrial ecosystems, almost all plants intimately associate with rhizospheric microorganisms (Lahrmann et al. 2013). These microorganisms such as mycorrhizal fungi, symbiotic nitrogen-fixing bacteria and some other free living plant growth promoting rhizobacteria (PGPR) are well known to contribute to soil fertility and crop production (Malla et al. 2002; Prasad et al. 2015). Mycorrhizal fungi and nitrogen fixing rhizospheric bacterial associations with plants are some of the best explored examples of mutualistic symbiosis (Prasad et al. 2013).

AM fungi are the key components of soil microbiota and the regulation of mycorrhizal formation is influenced by soil microorganisms (Saranya and Kumutha 2011). The root inhabiting endophytic fungus *P. indica* interacts with diverse group of microorganisms including *Sebacina vermifera, Pseudomonas fluorescens, Chlamydomonas reinhardtii, Aspergillus niger, Rhizopus stolonifer*, *Azotobacter chroococcum, Azospirillium brasilensis, Bradyrhizobium* spp (Pham et al. 2004; Gill et al. 2016). Interaction between mycorrhizal fungi and soil microorganisms involve nutrient cycling with impact on plant growth and nutrition. Microbial compounds produced by soil microorganisms that increase root cell permeability such as plant hormones are involved in the formation of symbiosis. The root exudates modified by the mycorrhizal condition, in turn, affect soil microbial population (Sailo and Bagyaraj 2006).

Sometimes, the bacteria and mycorrhizal fungi interact synergistically to mobilize soil PO_4 to the plant root through solubilization or mineralization. Occasionally, specific rhizobacteria are also known to affect the pre-symbiotic stages of AM development. This highly specific host–microbe interaction might be due to active molecular communication and physiological association among microbial multitropic communities. However, different interactions among rhizospheric microorganisms are also crucial for the development of sustainable strategies for soil fertility and crop production (Serfling et al. 2007).

The combined inoculation of AM fungi and diazotrophs has been found to increase the growth, nutrient uptake of plant nutrients, dry weight and yield on wide variety of agricultural and horticultural crops (Ansari et al. 2014). Selection of AM fungi strains for the improvement of crop yields and diazotrophs efficiency should consider inter-symbiont compatibility in addition to host plant compatibility. Rhizospheric microorganisms like *P. indica* and *A. chroococcum* are well known for their beneficial interaction with plants (Bhuyan et al. 2015).

18.3 Symbiotic Relationship Between *P. indica* and Rhizospheric Microorganism Improves Growth and Development of Plants

AM symbiosis with microorganisms is often associated with improved plant growth. This enhanced growth has been attributed to nutritional and non-nutritional effects of AM fungi. Interaction benefit plants by increasing the uptake of nutrients such as N, P, Zn and Cu (Das et al. 2014). The non-nutritional effects of mycorrhizae would be due to increased tolerance to saline conditions, improved water relations, increased survival rate of transplanted seedlings, control of root diseases and increased soil aggregation by the external hyphal network (Dodd and Thomson 1994). The mycorrhizal fungi are also known to produce wide array of plant growth promoting substances like Indole acetic acid (IAA), Indole butyric acid (IBA), Giberrilic acid (GA) (Saranya and Kumutha 2011). Plants with co-inoculation of AM fungi with other synergistic microbes like PGPR, mainly improved the growth and recorded increased yield of crop plants like rice, groundnut, maize, mulberry, banana, pepper, nutmeg, clove, cardamom, papaya, trifoliate orange, onion, tapioca, sweet potato, tomato, moringa, gourds and other flower crops (Andrade-Linares et al. 2013). Thus, the AM symbiosis play a significant role in ensuring increased plant growth and yield by their synergistic interactions with different groups of microbes like N_2 fixers, P mobilizers, bio-control agents, etc. (Sailo and Bagyaraj 2006; Saranya and Kumutha 2011).

The endogenous content of N, P and K was reported to be higher in chickpea and black lentil upon *P. indica* colonization, leading to better growth performance of plants (Nautiyal et al. 2010; Ansari et al. 2014). Inoculation of *P. indica* and *R. leguminosarum* to *Phaseolus vulgaris*, in addition to vermicompost treatments,

resulted increase in length and dry weight of roots and shoots (Tuladhar et al. 2013). The colonization of *P. indica* has been reported to significantly increase the germination and formation of seed in various plants including *Oryza sativa, Zea mays, Tridax procumbans, Nicotiana tabacum, Arabidopsis thaliana* and *Brassica oleracea* var *capitata* (Ansari et al. 2014). Additionally, *P. indica* also promotes the seed germination in leafy vegetables viz., cabbage, endive, Swiss chard (palak), red radish, onion, carrot, cauliflower, beetroot, pea and snow pea under extremely low temperatures (Serfling et al. 2007; Varma et al. 2012).

P. indica induces antioxidant system via CAS protein in *Brassica campestris* subsp. c*hinensis* leaves to provide dependable growth under drought (Sun et al. 2010). The AM fungi in coordination with soil microorganisms was found to defend tomato plants to confer with the ability to undergo vegetative and generative development under biotic stress (Andrade-Linares et al. 2013). A stable growth profile has been observed in *P. indica* colonized plants even under adverse environmental conditions acting as bio-control fungus (Varma et al. 2012; Ansari et al. 2014).

AM are soil fungi having symbiotic association with higher plants and it has been proven that *P. indica* is involved in P uptake to the host plants (Kumar et al. 2012). Investigations on wheat plants inoculated with *P. indica* revealed that gene expression level of phosphate transporter of the fungi was higher especially under P deficit condition (Gill et al. 2016). Another study with co-inoculation of a phosphate solubilizing bacterium *Pseudomonas striata* and *P. indica* showed a synergistic effect on chickpea by enhancing nodulation and growth (Nautiyal et al. 2010). These studies reflect the basis of different interactions of *P. indica* with rhizospheric bacteria for use in agronomical application.

A. chroococcum is a free living bacterium known to improve plant growth either through nitrogen fixation or other plant growth promoting traits. Single inoculants of *A. chroococcum* were found to enhance the growth of bamboo shoots and maize plants by phosphate solubilization and phytohormone production. Dual inoculants, in the presence *Glomus fasciculatum* (AM fungus), significantly enhanced the growth of tomato plants when compared with plants colonized by *G. fasciculatum* alone, while *A. chroococcum* enhanced root colonization and spore production by the mycorrhizal fungus. This mycorrhizal infection profoundly increased *A. chroococcum* population (Bhuyan et al. 2015).

18.4 Vermicompost Influences Symbiotic Activity of *P. indica* and *A. chroococcum*

Vermicompost is produced by biodegradation of organic matter through the combined activity of earthworm and microorganisms. It improves the texture and property of soil being rich in nitrogen, phosphorus and potassium (NPK) and important plant growth hormones (Tuladhar et al. 2013). Composts has been

recognized generally as an effective means for improving soil aggregation, structure and fertility, increasing microbial diversity and populations, improving the moisture-holding capacity of soils, increasing the soil cation exchange capacity (CEC) and thus increasing crop yields. Vermicompost contains most nutrients in plant-available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium influencing the growth and productivity of plants significantly (Azarmi et al. 2008). The addition of vermicompost in tomato plant had significant positive effect on soil chemical and physical properties (Madani et al. 2013).

The experiment was conducted in sterilized soil filled earthen pot. Vermicompost was initially added to soil. Ten surface sterilized healthy seeds of *Oryza sativa* were sown in each pot at a depth of 2 cm of soil and left for 7 days for germination. *P. indica* inoculum prepared by cutting 4 mm diameter agar culture disc from 1 week old culture was carefully placed near root surface. After 5 days of *P. indica* inoculation, 1 ml *Azotobacter* broth with inoculum size of 1.6×10^7 cfu/ml was applied to each plant pot. Five different treatments were used in the experiment which were control plants without any inoculation, plants inoculated with vermicompost only, plants inoculated with *A. chroococcum* alone, plants inoculated with *P. indica* alone and plants inoculated with *P. indica, A. chroococcum* and vermicompost. Growth parameters like shoot length, root length, fresh and dry shoot weight, fresh and dry root weight and panicle number were measured after cultivation on 45th day for vegetative stage and 90th day for reproductive stage. Rice plants treated with vermicompost showed significant improvement in growth over the untreated control plants in both vegetative and reproductive stages (Tables 18.1 and 18.2).

Table 18.1 Effects of treatments on growth parameters of rice plant at vegetative stage

Treatment	Shoot length[a] (cm)	Root length[a] (cm)	Fresh shoot wt.[a] (g)	Dry shoot wt.[a] (g)	Fresh root wt.[a] (g)	Dry root wt.[a] (g)
Control	31.3	10.7	0.7	0.2	0.2	0.1
T1	35.6	14.8	1.2	0.3	0.4	0.1
T2	35.0	13.7	0.8	0.2	0.4	0.1
T3	36.7	15.4	1.0	0.4	0.8	0.2
T4	37.4	17.1	1.7	0.7	0.9	0.3
Mean	35.2	14.3	1.1	0.4	0.5	0.1
LSD at 0.05	2.2	0.95	0.1	0.1	0.2	0.09

T1, vermicompost only; T2, *A. chroococcum* only; T3, *P. indica* only; T4, *P. indica, A. chroococcum* and vermicompost

[a]Data represents mean of six replicates

Table 18.2 Effects of treatment on growth parameters of rice plant at reproductive stage

Treatment	Shoot length[a] (cm)	Root length[a] (cm)	Fresh shoot wt.[a] (g)	Dry shoot wt.[a] (g)	Fresh root wt.[a] (g)	Dry root wt.[a] (g)	Panicle number
Control	100.7	35.2	23.3	16.4	19.8	10.3	0.8
T1	101.2	35.3	24.2	18.0	26.2	10.3	2.6
T2	100.9	35.2	24.1	16.6	20.1	8.9	2.4
T3	101.2	37.9	29.1	18.6	23.3	10.3	2.7
T4	107.2	39.6	32.3	23.4	38.5	16.9	3.6
Mean	102.2	36.6	26.6	18.6	25.6	11.2	2.4
LSD at 0.05	2.0	2.2	1.6	0.7	2.4	0.97	0.3

T1, vermicompost only; T2, *A. chroococcum* only; T3, *P. indica* only; T4, *P. indica, A. chroococcum* and vermicompost

[a]Data represents mean of six replicates

18.5 Effect of *Azotobacter chroococcum*, *Piriformospora indica* and Vermicompost on Growth of Rice Plant

The underground root system of plant is under the direct influence of diverse group of microorganisms. A significant positive response was observed with single inoculation of *A. chroococcum* in all growth parameters of reproductive stage attributing to the ability of nitrogen fixation except fresh root weight (Table 18.1). Nitrogen is the most important nutrient input required for rice production. Free living diazotrophs like *A. chroococcum* are very important biological nitrogen fixing microorganism. Nitrogen is usually the nutrient that limits plant production in wet lands and under nitrogen limited condition, plant roots excrete compounds with high C/N ratio, favoring rhizospheric nitrogen fixation (Klein et al. 1990). Increased growth in terms of leaf area index and tiller number/plant in rice plant was observed from *Azotobacter* application both individually and in combination with the use of organic and chemical fertilizers. Similarly, wheat seed inoculation with *A. chroococcum* strains significantly influenced the growth and yield (Barik and Goswami 2003). In the pot culture trial, rice plants inoculated with *P. indica* showed the significant beneficial effect in all the growth parameters of both vegetative stage (Table 18.1 and Fig. 18.1) and reproductive stage (Table 18.2 and Fig. 18.2) over the uninoculated control plants than *A. chroococcum* and vermicompost treatment. Maize, another important cereal crop was reported to produce more root and shoot biomass than control plants when inoculated with *P. indica* (Singh et al. 2003). Waller et al. (2005) studied the interaction of *P. indica* in monocotyledonous plants by establishing *P. indica* barley system in the laboratory. Infestation of barley roots with *P. indica* led to growth promotion and a modulation of resistance not only in the roots, but also in the leaf.

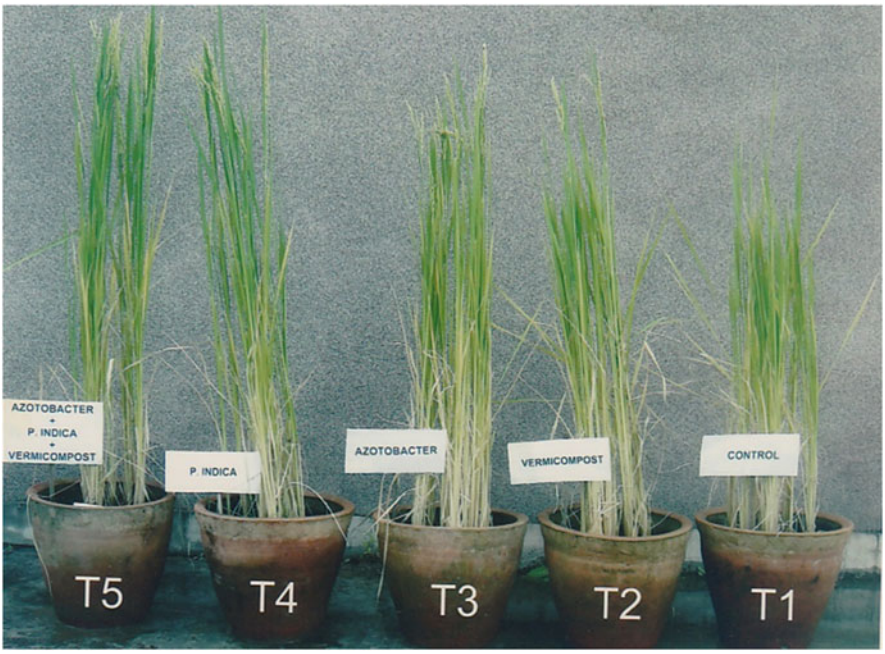


Fig. 18.1 Effects of treatments on growth parameters of rice plant at vegetative stage. *T1* control; *T2* vermicompost only; *T3 A. chroococcum* only; *T4 P. indica* only; *T5 P. indica, A. chroococcum* and vermicompost

18.6 Dual Inoculation of *P. indica* and *A. chroococcum* Affects Nitrogen, Phosphorus and Potassium (NPK) Content of Rice Plant

Use of AM fungi and nitrogen fixing bacteria as single inoculants and in combination have the ability to increase NPK as well as other nutrients in inoculated plants. The NPK content of plant was found to be highest in dual inoculated plants along with vermicompost in all the treatments than the untreated control plant in vegetative stage (Fig. 18.3) and reproductive stage (Fig. 18.4).

Beneficial effect on host plant as a result of mycorrhizal infection is usually associated with improved plant nutrition, especially phosphorus by virtue of extensive root system that extend the functional mycelium into surrounding soil, making a greater pool of nutrients available to the plant. This leads to increased plant growth, often as high as several hundred-fold increases in biomass (Vadakattu and Paterson 2006). *P. indica* has been found to mediate phosphorus uptake from the medium and translocate it to the host in an energy dependent process by producing significant amount of acid phosphates for the mobilization of broad range of insoluble forms of phosphates, enabling the host plant the accessibility of adequate phosphorus from immobilized reserves in the soil (Varma et al. 2001). Similarly,

Fig. 18.2 Effects of treatments on growth parameters of rice plant at reproductive stage. *T1* control; *T2* vermicompost only; *T3 A. chroococcum* only; *T4 P. indica* only; *T5 P. indica, A. chroococcum* and vermicompost

the experiment showed that dual inoculation of *P. indica* and *A. chroococcum* had effect on pH, NPK and organic content of soil treated with vermicompost. The pH of post- harvested soil of all treatments were lower than pre-cultivated soil suggesting that uptake of nutrients lead to release of H^+ into soil to compensate for excess cation uptake (Hinsinger et al. 2003). The uptake of nitrogen in the form of ammonium ion causes rhizospheric acidification in soil treated with *A. chroococcum* either in single or in combination of *P. indica* (Fig. 18.5). The absorption of nutrients by plants resulted in increase of phosphorus and potassium content of precultivated soil than post harvested soil (Fig. 18.6). The organic content was highest with soil receiving vermicompost treatment.

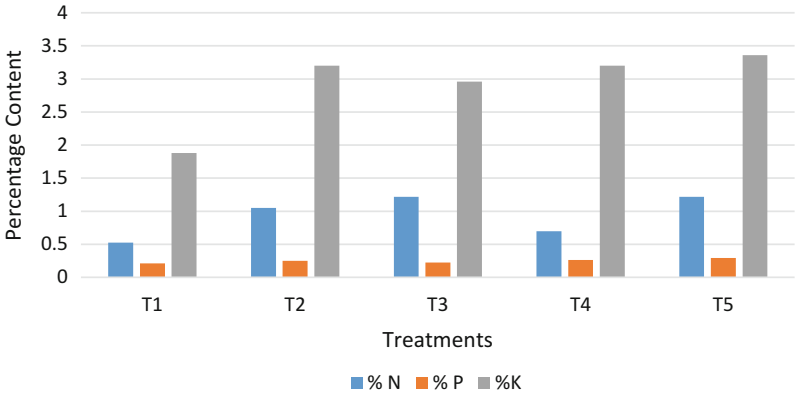


Fig. 18.3 Percentage of NPK content in plant in different treatments at vegetative stage. *T1* vermicompost only; *T2 A. chroococcum* only; *T3 P. indica* only; *T4 P. indica, A. chroococcum* and vermicompost

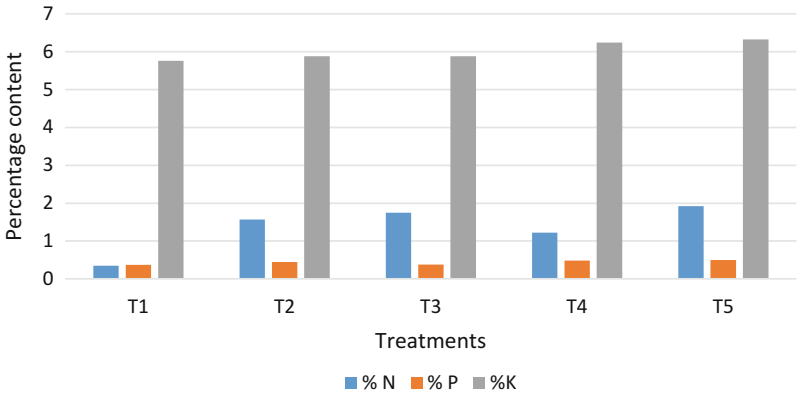


Fig. 18.4 Percentage of NPK content in plant in different treatments at reproductive stage. *T1* vermicompost only; *T2 A. chroococcum* only; *T3 P. indica* only; *T4 P. indica, A. chroococcum* and vermicompost

18.7 Conclusion

The inoculation of plant with rhizospheric organism along with AM fungi enhances overall growth performances in vegetative and reproductive stage resulting in increase of NPK content. These features make *P. indica, A. chroococcum* co-inoculation of crops most promising with respect to sustainable agriculture. The synergistic interaction of AM fungi with beneficial soil microbes can be better utilized in the future to promote stable returns from agricultural point of view by eliminating crop failure.

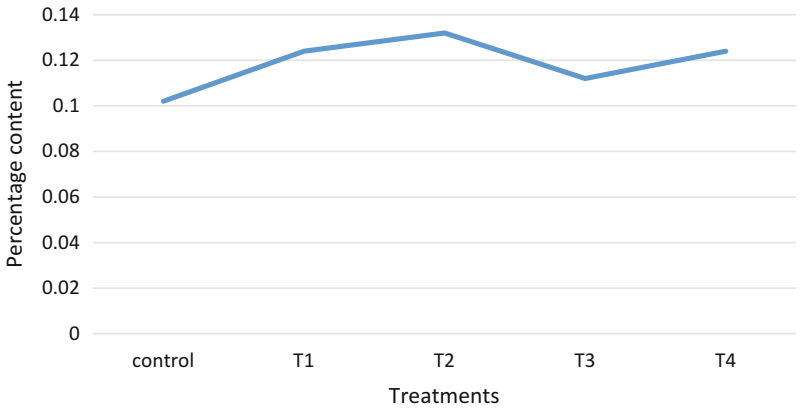


Fig. 18.5 Percentage of nitrogen content in soil in different treatments. *T1* vermicompost only; *T2 A. chroococcum* only; *T3 P. indica* only; *T4 P. indica, A. chroococcum* and vermicompost

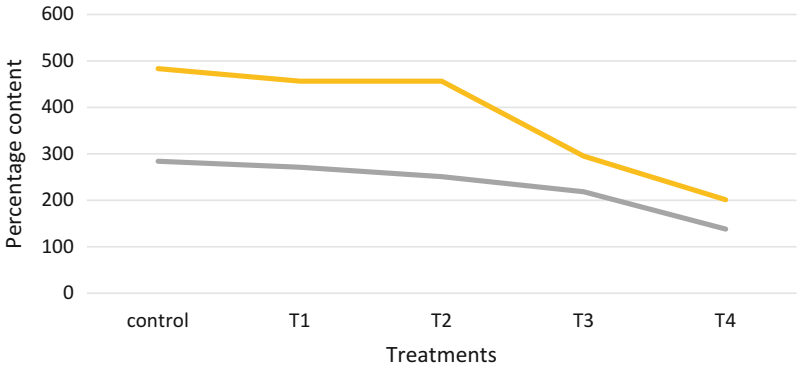


Fig. 18.6 Percentage of phosphorus and potassium content in soil in different treatments. *T1* vermicompost only; *T2 A. chroococcum* only; *T3 P. indica* only; *T4 P. indica, A. chroococcum* and vermicompost

References

Andrade-Linares DR, Müller A, Fakhro A, Schwarz D, Franken P, Ahmad F, Schwarz D, Franken P (2013) Impact of *Piriformospora indica* on tomato. In *Piriformospora indica:* Sebacinales and their biotechnological applications. Soil biology, vol 33, pp 107–117

Ansari MW, Gill SS, Tuteja N (2014) *Piriformospora indica* a powerful tool for crop improvement. Proc Indian Natl Sci Acad 80:317–324

Azarmi R, Giglou M, Taleshmikail R (2008) Influence of vermicompost on soil chemical and physical properties in tomato (*Lycopersicum esculentum*) field. Afr J Biotechnol 7:2397–2401

Barik AK, Goswami A (2003) Efficacy of biofertilizers wiith nitrogen levels on growth, productivity and economics in wheat (*Triticum aestivum*). Indian J Agron 48:100–102

Bhuyan SK, Bandyopadhyay P, Kumar P, Kumar Mishra D, Prasad R, Kumari A, Upadhyaya K, Varma A, Yadava P (2015) Interaction of *Piriformospora indica* with *Azotobacter chroococcum*. Sci Rep 5:13911. https://doi.org/10.1038/srep13911

Cruz C, Fegghi Z, Martins-Loução MA, Varma A (2013) Plant nitrogen use efficiency may be improved through symbiosis with *Piriformospora indica*. In Soil biology. *Piriformospora indica*. Sebacinales and their biotechnological applications, vol 33, pp 285–293

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

Deacon J (2006) The nitrogen cycle and nitrogen fixation. In The microbial world: the nitrogen cycle and nitrogen fixation. Jim Institute of Cell and Molecular Biology, The University of Edinburgh, pp 569–595

Declerck S, Strullu DG, Fortin A (2005) In vitro culture of Mycorrhizas. In: Soil biology, vol 4. Springer, Berlin, Heidelberg, pp 3–14

Dodd JC, Thomson B (1994) The screening and selection of inoculant arbuscular-mycorrhizal and ectomycorrhizal fungi. Plant Soil 159:149–158

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari A, Johri A, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:1–20

Hinsinger P, Plassard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59

Klein DA, Salzwedel JL, Dazzo F (1990) Microbial colonization of plant roots. In: Biotechnology of plant-microbe interaction. McGraw Hill Publishing, New York, pp 189–218

Kumar M, Sridevi K, Tamilarasan R (2012) Assessment of cadmium and its impact on the uptake efficiency of phosphate fertilizers by *Amaranthus tricolour*. J Mater Environ Sci 3:947–954

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 USA 110:13965–13970

Madani H, Shishehbor M, Madani H, Ardakani MR (2013) Effect of vermicompost and biofertilizers on yield and yield components of common millet (*Panicum miliaceum*). Ann Biol Res 4:174–180

Malla R, Singh A, Md Z, Yadav V, Suniti, Verma A, Rai MV (2002) *Piriformospora indica* and plant growth promoting Rhizobacteria: an appraisal. In Frontiers of fungal diversity in India, pp 401–419

Nautiyal CS, Chauhan PS, DasGupta 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

Pham GH, Kumari R, Singh A, Malla R, Prasad R, Sachdev M, Kaldorf M, Buscot F, Oelmüller R, Hampp R, Saxena AK, Rexer K-H, Kost G, Varma A (2004) Axenic cultures of *Piriformospora indica*. In: Plant surface microbiology. Springer, Germany, pp 593–613

Prajapati K, Yami KD, Singh A (2010) Plant growth promotional effect of *Azotobacter chroococcum*, *Piriformospora indica* and vermicompost on rice plant. Nepal J Sci Technol 9:85–90

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 (PGPR) and medicinal plants. Springer International Publishing, Switzerland, pp 247–260

Sailo GL, Bagyaraj D (2006) Influence of *Glomus bagyarajii* and PGPRs on the growth, nutrition and forskolin concentration of *Coleus forskohlii*. Biol Agric Hortic 23:371–381

Saranya K, Kumutha K (2011) Synergistic interactions of AM fungi with essential groups of microbes in plant growth promotion. Int J Curr Res 3:26–30

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

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 A, Singh A, Kumari M, Rai MK, Varma A (2003) Biotechnological importance of *Piriformospora indica Verma et al*—a novel symbiotic mycorrhiza-like fungus: an overview. Special issue on plant molecular biology and biotechnology. National Institute of Science Communication and Information Resources, New Delhi, India. Indian J Biotechnol 2:65–75

Sun C, Johanson CM, Cai D, Sherameti I, Oelmuller R, Loi B (2010) *Piriformospora indica* confers drought tolerance in chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017

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, Kost G, Oelmüller R (eds) *Piriformospora indica*. Soil biology, vol 33. Springer, Berlin, Heidelberg, pp 191–199

Vadakattu G, Paterson J (2006) Free living bacteria lift soil nitrogen supply. Farm Ahead 169:40

Varma A, Verma S, Sudha Sahay N, 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 Sahay NS, Sharma J, Roy A, Kumari M, Rana D, Thakran S, Deka D, Bharati K, Franken P, Hurek T, Blechert O, Rexer KH, Kost HA, Hock B, Maier W, Walter M, Strack D, Kranner I (2001) *Piriformospora indica:* a cultivable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) The mycota IX. Springer, Berlin, pp 125–150

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 IX, 2nd edn. Springer, Berlin, Heidelberg, pp 231–254

Varma A, Fekete A, Srivastava A, Saxena AK, Frommberger M, Li D, Gschwendter S, Sherameti I, Oelmueller R, Schmitt-Kopplin P, Tripathi S (2013) Inhibitory interactions of rhizobacteria with the symbiotic fungus *Piriformospora indica*. In: *Piriformospora indica* vol 33. Springer, Berlin, Heidelberg, pp 201–219

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heoer T, HuCkelhoven R, Neumann C, Wettstein DV, Franken P, Kogel K-H (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci USA 102:13386–13391

Chapter 19
Principles and Application of Confocal Microscopy to Understand Symbiotic Fungi

Surbhi Dabral, Diksha Bhola, Manpreet Kaur Attri, Manoj Nath, Ram Prasad, Saumya Singh, and Ajit Varma

Abstract *Piriformospora indica* is an endophytic fungus of the Sebacinaceae family that colonizes the roots of a variety of plant species. As a result of this plant fungus association, plant benefits with respect to nutrient acquisition, resistance against biotic and tolerance to abiotic stress. The fungal hyphae form chlamydospores after entering into the root cortex. Confocal microscope can be used for the detailed analysis of the fungal chlamydospores, its ultrastructure, morphology, sporulation, germination. Confocal microscopy captures the high resolution image of living as well as dead cells. This instrument helps to take three dimensional image of the objects as it eliminates out of focus glare by filtering the laser light along with confocal pinhole and excitation pinhole in front of detector, whereas in other microscopy techniques the entire sample is illuminated, including the area adjoining the area of interest which interferes with the analysis. The basic features which make confocal microscopy better than other microscopy techniques are removal of out of focus glare, shallow depth of field, optional sectioning, volume analysis, live cell imaging and lambda scanning. *P. indica* is grown and also the culture is maintained on Hill and Kaefer media at pH 6.5 and 30 °C. Batch as well as continuous fermentation can be used for the production of fungal biomass and spores.

19.1 Introduction

Fast and reliable *in situ* imaging of biological species remains a long-standing goal in photonics to understand dynamic procedures including complex multicellular organisms. The fluorescence imaging delivers point-by-point construction of images in a volumetric space and has been broadly used to monitor complex cellular events (Prabhat et al. 2004; Siddhanta et al. 2017). Additionally, recent advances in super-resolution microscopy have revealed a new opportunities to track

S. Dabral • D. Bhola • M.K. Attri • M. Nath • R. Prasad • S. Singh • A. Varma (✉)
Amity Institute of Microbial Technology, Amity University, E-3, Fourth Floor, Sector 125, Noida 201313, India
e-mail: ajitvarma@amity.edu

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_19

molecular dynamics in unique detail (Fernandez-Suarez and Ting 2008). The recent advancement of confocal microscopy and its application to study the physiological and metabolic changes in microbes–microbes, plant–pathogen interactions have revolutionized research into the character of selected biomolecules and cell machineries in pathogen infection strategies, identifying special molecular mechanisms (gene expression) and plant defense responses. Confocal microscopy allows high-resolution visualization of a variety of fluorescent and fluorescently tagged molecules in both fixed and living cells, not only in single cells but also in intact tissues. Confocal microscopes greatly improve image quality by reducing interference by out-of-focus light and can capture high-resolution optical images through samples in the z-axis. In combination with a range of computational image analysis techniques, confocal microscopy provides a potent tool by which molecules, earlier detection and characterization of disease, molecular interactions, and cell components can be localized and studied (Massoud and Gambhir 2003; Hardham 2012).

Piriformospora indica is an endophytic fungus with a wide range of higher plants and provides multifaceted amenities such as nutrient uptake, disease resistance, abiotic and biotic stress tolerance and growth promotional including value additions (Varma et al. 2012; Prasad et al. 2008, 2013; Gill et al. 2016). The hyphae of the fungus can enter into the root cortex and form chlamydospores. After culturing of fungus on synthetic media, we achieved confocal microscopy to analyze the form and structure of the hyphae and chlamydospores (Siddhanta et al. 2017). The hyphae are straight and hyaline, and the surface of the hyphal walls is smooth. The chlamydospores are pear shaped and have smooth walls. *P. indica* biotrophic colonization pattern can be accompanied by abroad-spectrum suppression of root innate immunity (Qiang et al. 2011). In the support of the large host range of *P. indica*, molecular and genetic analyses revealed that plant roots, similar to leaves, are equipped with an effective innate immune system where immune suppression by *P. indica* was considered as a prerequisite for successful root colonization (Qiang et al. 2011; Gill et al. 2016).

19.2 Principle and Application of Confocal Microscopy

Microscopy is a very important tool to study microorganisms, the revolution came when Anton van Leeuwenhoek (1675) observed microbes using a handcrafted microscope. Since, then enormous modifications have been made in this technique ranging from simple light microscope to highly advanced confocal and super resolution microscopy. The first ever microscope was developed by Zacharias and Hans Janssen in 1590. Later in the year, 1667 Robert Hooke made many changes in the compound microscope and published the famous “Micrographia”. In the year, 1675 Anton von Leeuwenhoek developed a simple microscope to observe bacteria and protozoans. Although he was not the first person to develop a microscope, but his was the best of the period. In the twentieth century, the resolution limit of visible light was overcome by UV light microscope. In 1931,

Knoll and Ruska developed the first transmission electron microscope. Later, TEM was improved by Ruska to form first Scanning Electron microscope. In 1988, Marvin Minsky, developed the first confocal scanning microscope. Later, first commercialized confocal scanning microscope was developed by Czechoslovak Mojmir Petran of Charles University in Plzen developed the Tandem Scanning Microscope, which was the first ever commercialized confocal microscope. Time to time many changes were made in the concept of confocal microscope according to the need of the experiment.

The technique has made imaging of cellular interactions, ultrastructure and morphology of cells possible which can help the scientists for doing better research leading to a variety of agricultural and biotechnological advancements (Ahmad and Khan 2012).

Imaging is a very powerful tool to view filamentous microbes such as fungi where morphology is a very important aspect from industrial point of view as directly or indirectly it is related to fermentation, be it's hyphal structures or spores, microscope plays a very important role in fungal research (Czymmek et al. 1994). *P. indica* is a plant growth promoting mycorrhiza-like fungus which helps in alleviating stress conditions in plants. It has shown to combat various abiotic and biotic stress in different plants hence the study of this fungi at microscopic level is essential (Varma et al. 2012). With the help of various staining methods fungal structures can be observed in roots which can help in interpretation of results. Roots of plants/fungus can directly be observed under bright field microscope to get a general overview and this method still remains the standard for root colonization studies. In depth analysis will require sophisticated and advance microscopy techniques. Confocal laser microscopy is highly efficient technique for researchers interested in better imaging and analysis of cell structure and function. With the help of reliable data accurate interpretations can be done. The present book chapter aims to give a brief description about confocal microscope (Fig. 19.1a).

Marvin Minsky in 1988 desired to image neural networks in living brain which drove him to invent confocal microscope. The technique was patented in 1957. Three dimensional images of biological and non-biological samples is possible by this instrument as it eliminates out of focus glare by filtering the laser light along with confocal pinhole and excitation pinhole in front of detector. Advanced optics along with transverse resolution results in high quality images. The laser intensity is reduced due to the presence of pinhole, to overcome this lasers are coupled with optical fibers so that increase in number of excitation wavelengths can give a bright, clear and haze free image. A 3D image can be produced with the help of powerful softwares (Singh et al. 1998). Removal of out of focus glare, shallow depth of field, optical sectioning, volume analysis, fluorescence recovery after photobleaching, förster resonance energy transfer, live cell imaging, lambda scanning etc., are some of the basic features which make this above all the others conventional microscopic techniques. Cells weather live or dead need to be stained with specific fluorescent probes. In conventional microscopes the samples when hit with a light of specific wavelength emits fluorescence, apart from the region of interest other areas also gets illuminated which interferes with the resolution of the specimen. Frequent problem occurs with samples

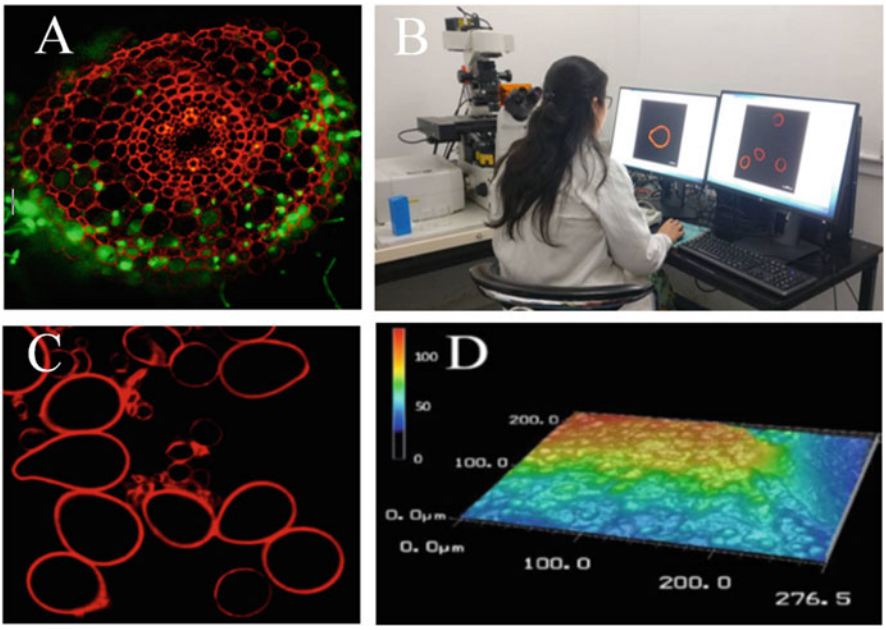


Fig. 19.1 (**a**) A confocal view of mycorrhized root, (**b**) Nikon Confocal A1, (**c**) *Piriformospora indica* spores viewed in Nikon Confocal A1 microscope, (**d**) CLSM image exhibiting the uneven and complex surface topology of a section of the fungal culture [reprinted with permission from reference Siddhanta et al. (2017), Copyright 2017 John Wiley & Sons]

having thickness >2 μm, secondary fluorescence emitted by the sample interferes with the resolution. Confocal laser scanning microscopes can solve this problem as the instrument has the ability to eliminate out of focus light and scanning done through lasers provides axial and lateral resolution thus giving a clear picture of the specimen. Talking about resolution and not mentioning electron microscope will be injustice to the most advanced microscopic techniques. In electron microscope the accelerated beam of electrons is used for acquiring image. Wavelength of electrons is much shorter than photons that's why the resolution is too high as compared to confocal and other conventional microscopy techniques. There is one major limitation with electron microscopy we can not observe live samples as we can do in confocal. This limits our studies only to dead samples whereas in confocal we can view the live samples for hours, even days with the help of time lapse and perfect focus system. We can say that confocal microscope has bridged the gap between the basic and advanced microscopy techniques (Fig 19.1b).

The question comes into mind how does confocal capture image which is sharp and haze free? In conventional microscope the whole sample is illuminated by light coming from a light source preferably a xenon or mercury lamp, this allows other areas adjacent to the region of interest to brighten up which interferes with the imaging of the interested point, we can directly see the image onto the eyepiece. However, in confocal microscopes the technique to image a sample is different. Here the laser source falls on the sample for illumination, it scans the specimen and

optical sections are produced. It is a non-invasive technique with which the focused light is used to section the specimen and instrument collect images in the form of optical sections. As the light source is laser we cannot see the specimen directly through eye piece, the signal produced by the sample is multiplied by photo multiplier tubes and the image can be viewed in computer (Amos and White 2003).

Now a days laser scanning confocal microscope is widely used in research, with modifications in the fluorescence microscope anyone can attain the benefits of this technique. It's simple design and user friendly approach has made it accessible to all. Sample preparation especially for confocal is not a problem as the protocol for both fluorescence and confocal is same. The difference lies that the instrument has laser as the light source which is coupled with the photomultiplier detectors, it multiplies the signal and a computer is attached to control all the scanning devices so that acquisition of the image can take place properly. Presence of pinhole is also a very important as it eliminates out of focus light. We can say that the presence of laser light source, pinhole and various dichoric mirrors, photomultiplier detectors along with the objective lenses are responsible for acquisition of a clear optical sectioned image which can produce a 3d view of the region of interest. All these features are not present in fluorescence microscope. After the acquisition of image it can be stored in computer and various types of analysis can be done with the help of various softwares.

Now going into functioning illumination as well as detection are restricted to a single diffraction limited point of the sample. Objective lenses which are available from 10× to 100×, depending on what magnification the image is to be acquired brings the point of illumination to focus in the sample. This is scanned by the scanner which is attached to the computer where the acquired image can be seen. The signals of sequence of the scanned image known as optical sections are detected by photo multiplier tubes through a pinhole. PMT's multiply the signals coming through pinhole and the output is displayed in the computer. The specimens are labeled with the fluorescent probes or dyes, when laser light falls on the labeled samples the photons get excited move to higher energy shells, while coming back to their ground state they emit fluorescence. This signal is captured to produce images (Shinya 2006).

Confocal microscopy is an advanced technique which is constantly used in mycological research. Ultrastructures, morphology, sporulation, germination and host pathogen interaction studies plays a very important role to give an insight to a whole new level of advanced research which can help in achieving great discoveries (Fig. 19.1c) (Singhal et al. 2017).

19.3 A Case Study: Confocal Microscope Used for Fungal Studies

Lagopodi et al. (2002) used confocal laser scanning microscopy (CLSM) to study the behavior of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato root colonization (Fig. 19.2). *F. oxysporum* f. sp. *radicis-lycopersici* causes tomato

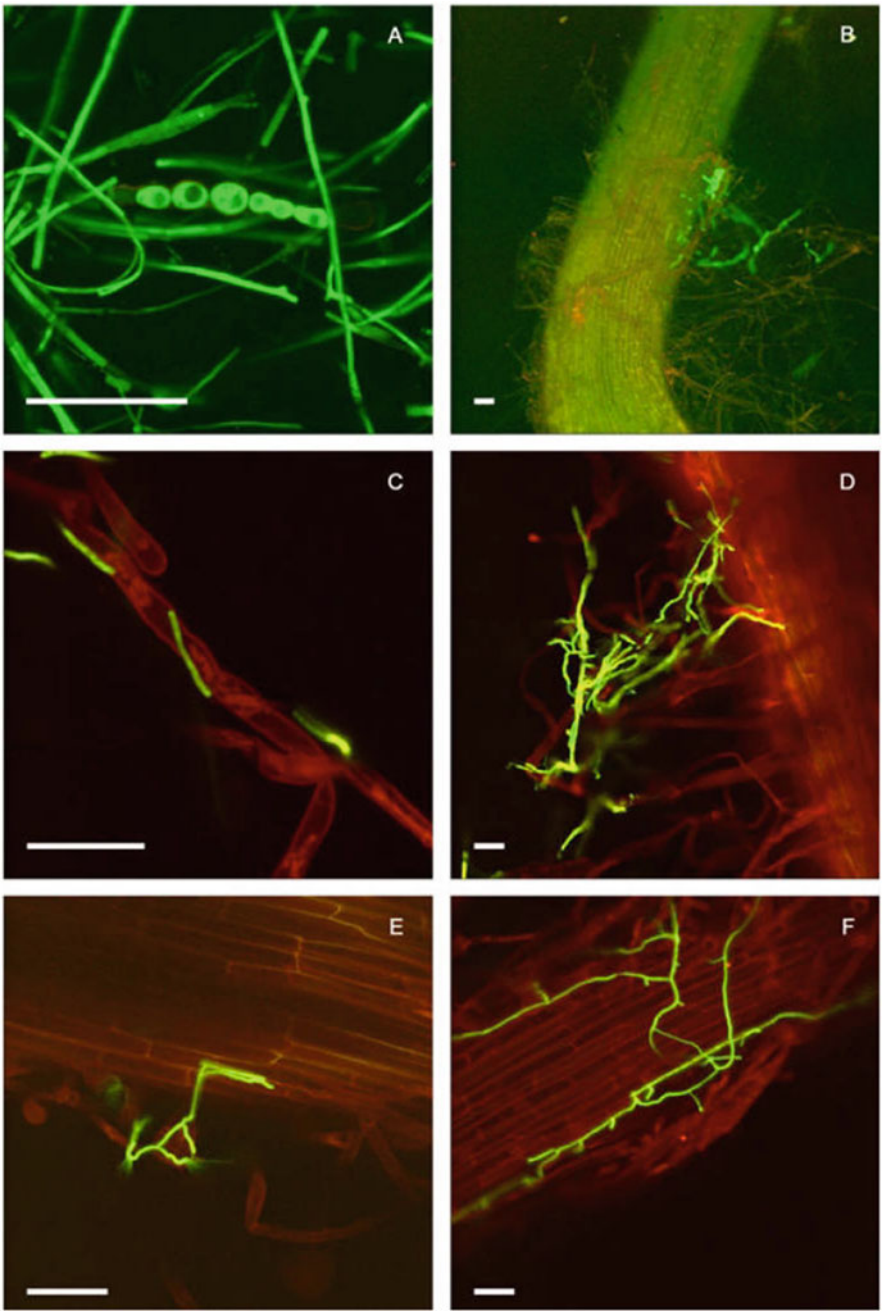


Fig. 19.2 Early stages of tomato root colonization by *Fusarium oxysporum f.* sp. *radicis-lycopersici* marked with gfp. Confocal scanning laser microscopy analyses of tomato roots grown after planting 2-day-old germinated sterile seedlings in sand containing spores *of*

foot and root rot disease. *F. oxysporum* is a soil fungus and is difficult to control. Green fluorescent protein from the jellyfish *Aequorea victoria* was used to label *F. oxysporum* f. sp. *radicis-lycopersici* in order to observe its presence and developmental stages in tomato. GFP's fluorescence is stable and does not depend on species. It also does not requires any substrate or cofactors for its reactions.

19.4 Plant and Microbe Interaction with Reference to *P. indica*

P. indica is a mycorrhiza like axenically cultivable plant growth-promoting root endophyte. It represents the order Sebacinales which is the elementary basidiomyceteous order projecting mycorrhizal capabilities (Matheny et al. 2007; Weiss et al. 2004, 2011). Further, *P. indica* which was formerly isolated from Thar Desert (Verma et al. 1998) is a biotroph and a model organism for investigational studies. *P. indica* is placed as a member of the Basidiomycetes order Sebacinales by the molecular phylogenetic analysis (Hibbett et al. 2007; Qiang et al. 2012; Weiss et al. 2004). The partial 18S rDNA sequence analysis placed *P. indica* in Basidiomycota close to the *Rhizoctonia solani* group (Varma et al. 2013a, b). A maximum likelihood analysis of 18S rDNA sequence confirmed these postulations. Further according to their similarities to Zygomycetes, *P. indica* is termed as an AM-like fungus (Franken et al. 2000). Leading further to the morphological traits of the fungus, *P. indica* has white to almost hyaline hyphae. The hyphae are thin walled and have a diametric range of 0.7–3.5 μm, irregularly septate and often exhibit anastomosis. The highly interwoven hyphae appear as intermingled cords and branch irregularly. External deposits, polysaccharides or hydrophobic proteins can be noticed on hyphal walls at regular intervals. The irregular septation of hyphae accounts for the presence of more than one nuclei in a single compartment. The distinct chlamydospores appear singly or in clusters. Initially the chlamydospores are thin walled and hyaline while they become thick walled towards maturity. Further no sexual structures or clamp connections were observed (Varma et al. 2001). The mycelium has a sub-surfaced and concentric growth on agar medium. When grown on solid culture media very few aerial hyphae were formed. Occasionally the mycelium fabricates periodic rings on agar medium, whereas the

Fig. 19.2 (continued) F. oxysporum f. sp. *radicis-lycopersici*. (**a**) Uniform expression of gfp in hyphae and chlamydospores of the transformed fungus grown on potato dextrose agar. (**b**) Fungal hyphae in contact with tomato root hairs, 2 days after inoculation. (**c**) Attachment of fungal hyphae to tomato root hairs, 2 days after inoculation. (**d**) Intermingling of hyphae with root hairs at the crown region, 3 days after inoculation. (**e**) Attachment of hyphae to the root surface and settling in the grooves between epidermal cells, 3 days after inoculation. (**f**) Colonization of the root surface by hyphae that are growing at the junctions of the epidermal cells, 3 days after inoculation. (**a–f**) *Scale bar* 50 μm (c.f. Lagopodi et al. 2002)

structure of the mycelium was homogenous. The morphological characters of the mycelium greatly differ with variations in conditions of cultivation or nutrient compositions of the culture medium. Readers are advised to see the article published by Weiß et al. (2016) have proposed the name Serendipitaceae for the family Sebacina and within it the genus Serendipita and have placed *Piriformospora indica* and *P. williamsii* (Weiß et al. 2016).

P. indica displays an endophytic lifestyle and have known to show symbiotic associations with majority of the terrestrial plants. It expansively colonizes the root hair zones inter and intracellularly while excluding the elongation and meristematic zones (Deshmukh et al. 2006). This pattern of colonization demarcates *P. indica* from ecto as well as arbuscular mycorrhizal fungi (AMF), which exclusively grow intercellularly or principally colonize the deeper cortex layers of younger roots (Smith and Read 2008). Symbiotic relationship of *P. indica* with various taxonomically unrelated hosts increases plant growth and biomass (Peškan-Berghöfer et al. 2004; Waller et al. 2005; Shahollari et al. 2007; Sherameti et al. 2008; Camehl et al. 2010, 2011; Hilbert et al. 2012; Nongbri et al. 2012; Lahrmann et al. 2013; Venus and Oelmüller 2013), higher seed yield, early flowering, and biotic and abiotic stress tolerance responses (Baltruschat et al. 2008; Singh et al. 2011). It has been reported that mutualistic associations of this fungus stimulates increased allocation of nutrients like phosphate to the plant roots (Yadav et al. 2010).

Since *P. indica* can be easily maintained and axenically cultured, it positions as an ideal models for beneficial fungus–plant interactions studies and has a promising perspective for application in sustainable horticulture and agriculture (Waller et al. 2005; Godoy et al. 2000; Kong et al. 2001; Ruan et al. 2011; Trivedi et al. 2012). Exploiting these plant benefitting properties, a formulation of *P. indica* with magnesium sulphite was prepared where magnesium sulphite acts as a carrier. For this, 2% (w/w) of fungal biomass served as effective and stable formulation. On an average the colony forming unit (CFU) count was maintained as 10^9 and moisture content was 20%. Application of this formulation on plants presented enhanced overall growth and resistance to biotic and abiotic stress.

19.4.1 Application of Piriformospora indica on Isabgol: A Case Study

Isabgol (*Plantago ovata*) are annual plant species that majorly grow in the arid and semi-arid regions and are extensively used in conventional and modern pharmacology (Patel et al. 1996). The seeds of blond psyllium are mainly valued for mucilaginous rosy white husk. The mucilage comprises of reserve carbohydrates mainly pantosans. The husk is commonly used for getting relief from constipation as per being a dietary fiber supplement acting as a bulk-forming laxative. It releases constipation through mechanically stimulating the intestinal peristalsis.

The seeds Isabgol (*Plantago ovata*) were treated with formulation of the AM fungi *P. indica* to study the effect on the growth and development of plant species. Nursery trails were conducted based on the season in the month of November. On application of *P. indica*, it was observed that the overall growth of the plant was promoted. The mean yield in Isabgol seed and husk respectively, increased to 57 and 33% g in *P. indica* treated seeds. There also was observed an early flowering in case of *P. indica* treated seeds.

19.5 Cultivation of *P. indica*

19.5.1 Culture Maintenance and Inoculum Preparation of P. indica

The culture of *P. indica* was maintained on Hill and Kaefer medium plates supplemented with 15 g/L agar. Plates were incubated at 30 ± 1 °C for 10 days and then stored at 4 °C (Prasad et al. 2005). For the preparation of inoculum, *P. indica* was initially grown on Kaefer medium in a petri dish and then transferred to the seed culture medium by punching out 8 mm of the agar plate culture with a sterilized cork-borer. The seed culture was grown in a 500 mL Erlenmeyer flask containing 100 mL potato dextrose broth at 30 ± 1 °C on a rotary shaker at 200 rpm for 4 days.

19.5.2 Cultivation of P. indica in Batch Culture

Batch culture is closed bacterial culture system with specific nutrient, temperature, pressure, aeration and other environmental conditions to optimise growth (Wilson 1995). Because nutrients are neither added, nor waste products removed during incubation, batch cultures can only complete a limited number of life cycles before nutrients are consumed and growth stops. In other words batch culture is a technique for large scale production of microbes or microbial products in which, at a given time, the fermenter is stopped and the culture is worked up. Cells, or products that the organisms have made, can then be harvested from the culture.

P. indica can be cultivated on Hill and Kaefer media under the optimized cultural conditions (inoculum size: 5%; agitation speed: 200 rpm; working volume: 50%; initial pH: 6.5; temperature: 30 °C) in 500 mL flask. The 500 mL flask containing 250 mL media was inoculated with 5% inoculum which was grown in a 500 mL flask containing 100 mL potato dextrose broth at 30 ± 1 °C on a rotary shaker at 200 rpm for 4 days. Now, keep this flask on rotary shaker at 200 rpm for 10 days (Fig. 19.3). In this method of cultivation maximum dry cell weight is

Fig. 19.3 (**a**) Typical view of a fermenter; (**b**) *P. indica* culture in fermenter

obtained after 5 days, the sporulation starts after 6 days of growth, and maximum spore yield is obtained after 8 days.

19.5.3 Cultivation of P. indica in Bioreactor

Continuous fermentation is a technique for production of microbes or microbial products in which nutrients are continuously supplied to the fermenter. The continuous culture of micro-organisms is a technique of increasing importance in microbiology. The essential feature of this technique is that microbial growth in a continuous culture takes place under steady-state conditions; that is, growth occurs at a constant rate and in a constant environment. Such factors as pH value, concentrations of nutrients, metabolic products and oxygen, which inevitably change during the 'growth cycle' of a batch culture, are all maintained constant in a continuous culture; moreover, they may be independently controlled by the experimenter. These features of the continuous culture technique make it a valuable research tool, while it offers many advantages, in the form of more economical production techniques, to the industrial microbiologist.

P. indica was cultivated in cylindrical stirred tank bioreactor of different capacity i.e. 3, 7 and 10 L (Fig. 19.3a) with a working volume of 50%. An agitator shaft with three Rushton flat blade turbine impellers was used for stirring. Each impeller consisted of six blades and the distance between two impellers was 8 cm (Khan et al. 2011). The orifice sparger was used for sparging air into fermentation broth. For all the bioreactor Bagde et al. (2010) experiments an active inoculum of 2% grown on potato dextrose broth for 4 days was used (Fig 19.3b).

19.5.3.1 Fermenter Sterilization

The fermenter vessel was filled with production media. Then the head plate consisting of the various probes and agitator module was fixed. The opening

pores like the sampling port and other opening pipes of the acid, alkali and antifoam supply was covered with cotton plugs. Finally the whole fermenter vessel along with the media inside was placed inside an autoclave and sterilized. The acid and the alkali were also autoclaved.

Spore count of inoculum used in above methods: The spore suspension was used as inoculum for fermentation (Kawaide 2006). The spore suspension was prepared by adding 10 mL of sterilized distilled water to the culture plates and then gently scraping the spores with the inoculation needle. Aqueous spore suspension consisting about 6×10^7 spores/mL was used (spores counted on Haemocytometer). The spore suspension was then transferred to another sterile empty test tube. Generally, 25 mL of the spore suspension was then inoculated into the fermenter vessel containing 3000 mL of the culture media.

However, when *P. indica* was grown in a 14-l bioreactor (Chemap AG, Switzerland) using Hill–Käfer medium maximum, dry cell weight was obtained after 42 h of growth, the fungus-initiated sporulation after 48 h, and a spore yield of 9.25×10^7 spores/mL was achieved after 60 h of growth. The early sporulation in this case may be due to rapid consumption of glucose. Owing 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 in the early sporulation compared with the batch culture in shake flasks.

19.6 Conclusion

Fermentation techniques are employed for increased production of *P. indica*, which can be commercially used for the enhancement of nutrient uptake by different plants or help them to sustain through different stress conditions. To establish different experiments for confirmed results for these conclusions, confocal microscopy has helped a lot along with other techniques. This microscopy technique helps us to have a detailed knowledge about the ultrastructure of the fungal hyphae, its spores, sporulation, it's germination and host pathogen interaction studies. It removes the background image disturbances which gives a clear image of the area of interest.

Acknowledgment Authors are thankful to DBT-SBIRI, DBT, and DST for providing confocal microscope facilities.

References

Ahmad I, Khan MSA (2012) Microscopy in mycological research with special reference to ultrastructures and biofilm studies. In: Mendez-Vilas A (ed) Current microscopy contributions to advances in science and technology. Formatex Research Center, Spain

Amos WB, White JG (2003) How the confocal laser scanning microscope entered biological research. Biol Cell 95:335–342. https://doi.org/10.1016/S0248-4900(03)00078-9

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 12:927–932

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel KH, Schaefer 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

Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J, Oelmüller R (2010) Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica*and *Arabidopsis thaliana*. New Phytol 185:1062–1072

Camehl I, Drzewiecki C, Vadassery J, Shahollari B, Sherameti I, Forzani C, Munnik T, Hirt H, Oelmüller R (2011) The OXI1 kinase pathway mediates *Piriformospora indica* induced growth promotion in *Arabidopsis*. PLoS Pathog 7:e1002051

Czymmek KJ, Whallon JH, Klomparens KL (1994) Confocal microscopy in mycological research. Exp Mycol 18:275–293

Deshmukh S, Hückelhoven 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 USA 103:18450–18457

Fernandez-Suarez M, Ting AY (2008) Fluorescent probes for super-resolution imaging in living cells. Nat Rev Mol Cell Biol 9:929–943

Franken P, Requena N, Bütehorn B, Krajinski F, Kuhn G, Lapopin L, Mann P, Rhody D, Stommel M (2000) Molecular analysis of the arbuscular mycorrhiza symbiosis. Arch Agron Soil Sci 45:271–286. https://doi.org/10.1080/03650340009366129

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW et al (2016) *Piriformospora indica*: potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

Godoy AV, Lazzaro AS, Casalongue CA, Segundo BS (2000) Expression of a *Solanum tuberosum* cyclophilin gene is regulated by fungal infection and abiotic stress conditions. Plant Sci 152:123–134

Hardham AR (2012) Confocal microscopy in plant-pathogen interactions. Methods Mol Biol 835:295–309

Hibbett DS, Binder M, Bischoff JF, Blackwell M, Cannon PF, Eriksson OE, Huhndorf S, James T, Kirk PM, Lücking R, Thorsten Lumbsch H, Lutzoni F, Matheny PB, McLaughlin DJ, Powell MJ, Redhead S, Schoch CL, Spatafora JW, Stalpers JA, Vilgalys R, Aime MC, Aptroot A, Bauer R, Begerow D, Benny GL, Castlebury LA, Crous PW, Dai YC, Gams W, Geiser DM, Griffith GW, Gueidan C, Hawksworth DL, Hestmark G, Hosaka K, Humber RA, Hyde KD, Ironside JE, Kõljalg U, Kurtzman CP, Larsson KH, Lichtwardt R, Longcore J, Miadlikowska J, Miller A, Mon-calvo JM, Mozley-Standridge S, Oberwinkler F, Parmasto E, Reeb V, Rogers JD, Roux C, Ryvarden L, Sampaio JP, Schüssler A, Sugiyama J, Thorn RG, Tibell L, Untereiner WA, Walker C, Wang Z, Weir A, Weiss M, White MM, Winka K, Yao YJ, Zhang N (2007) A higher-level phylogenetic classification of the fungi. Mycol Res 111:509–547

Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A, Hilbert M, Voll LM, Ding Y, Hofmann J, Sharma M, Zuccaro A (2012) Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol 196:520–534

Kawaide H (2006) Biochemical and molecular analysis of gibberellins biosynthesis in fungi. Biosci Biotechnol Biochem 70:583–590

Khan AL, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ (2011) Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, plant growth and isoflavone biosynthesis in soybean under salt stress. Process Biochem 46:440–447

Kong HY, Lee SC, Hwang BK (2001) Expression of pepper cyclophilin gene is differentially regulated during the pathogen infection and abiotic stress conditions. Physiol Mol Plant Pathol 59:189–199

Lagopodi AL, Ram AF, Lamers GE, Punt PJ, Van den Hondel CA, Lugtenberg BJ, Bloemberg GV (2002) Novel aspects of tomato root colonization and infection *by Fusarium oxysporum f. sp. radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Mol Plant Microbe Interact 15:172–179

Lahrmann U, Ding Y, Banhara A, Rath M, Hajirezaei MR, Döhlemann S (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. Proc Natl Acad Sci USA 110:13965–11397

Massoud TF, Gambhir SS (2003) Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev 17:545–580

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2007) Major clades of Agaricales: a multi-locus phylogenetic overview. Mycologia 98:984–997

Minsky M (1988) Memoir on inventing the confocal scanning microscope. Scanning 10:128–138

Nongbri PL, Johnson JM, Sherameti I, Glawischnig E, Halkier BA, Oelmüller R (2012) Indole-3-acetaldoxime-derived compounds restrict root colonization in the beneficial interaction between *Arabidopsis* roots and the endophyte *Piriformospora indica*. Mol Plant Microbe Interact 25:1186–1197

Patel BS, Patel JC, Sadaria SG (1996) Response of blond psyllium (*Plantago ovata*) to irrigation and phosphorus. Indian J Agron 41:311–314

Peškan-Berghöfer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanke V, Varma AK, Oelmüller R (2004) Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Physiol Plant 122:465–477

Prabhat P, Ram S, Ward ES, Ober RJ (2004) Simultaneous imaging of different focal planes in fluorescence microscopy for the study of cellular dynamics in three dimensions. NanoBiosci IEEE Trans 3:237–242

Prasad R, Pham GH, Kumari R, Singh A, Yadav V, Sachdev M, Peskan T, Hehl S, 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, Heidelberg, pp 291–312

Prasad R, Sharma M, Kamal S, Rai MK, Rawat AKS, Pushpangdan P, Varma A (2008) Interaction of *Piriformospora indica* with medicinal plants. In: Varma A (ed) Mycorrhiza, 3rd edn. Springer, Germany, 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

Qiang X, Weiss M, Kogel KH, Schäfer P (2011) *Piriformospora indica* – a mutualistic basidiomycete with an exceptionally large plant host range. Mol Plant Pathol 13:508–518

Qiang X, Zechmann B, Reitz MU, Kogel KH, Schafer P (2012) The mutualistic fungus *Piriformospora indica* colonizes *Arabidopsis* roots by inducing an endoplasmic reticulum stress-triggered caspase-dependent cell death. Plant Cell 24:794–809. https://doi.org/10.1105/tpc.111.093260

Ruan SL, Ma HS, Wang SH, Fu YP, Xin Y (2011) Proteomic identification of OsCYP2, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when over expressed. BMC Plant Biol 11:34. https://doi.org/10.1186/1471-2229-11-34

Shahollari B, Vadassery J, Varma A, Oelmüller R (2007) A leucine-rich repeat protein is required for growth promotion and enhanced seed production mediated by the endophytic fungus *Piriformospora indica* in *Arabidopsis thaliana*. Plant J 50:1–13

Sherameti I, Tripathi S, Varma A, Oelmueller 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

Shinya I (2006) Foundations of confocal sacanned imaging in light microscopy. In: Pawley J (ed) Handbook of biological confocal microscopy. Springer, Boston, MA, pp 1–19

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. Chem Phys Chem 18:72–78

Singh, Amit, Gopinathan KP (1998) Confocal microscopy: a powerful tool for biological research. Biology Faculty Publications Paper 120

Singh LP, Gill SS, Tuteja N (2011) Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signal Behav 6:175–191

Singhal U, Khanuja M, Prasad R, Varma A (2017) Impact of synergistic association of ZnO-nanorods and symbiotic fungus *Piriformospora indica* DSM 11827 on *Brassica oleracea* var. botrytis (Broccoli). Front Microbiol 8:1909. https://doi.org/10.3389/fmicb.2017.01909

Smith SE, Read DJ (2008) Mineral nutrition, toxic element accumulation and water relations of arbuscular mycorrhizal plants. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis, 3rd edn. Academic Press, London, pp 45–18

Trivedi DK, Bhatt H, Johri AK, Tuteja N, Bhavesh NS (2012) Sequence specific H, C and N NMR assignments of Cyclophilin A like protein from *Piriformospora indica* involved in salt stress tolerance. Biomol NMR Assign 7:175–178

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*: a cultivable mycorrhiza-like endosymbiotic fungus. In: Hock B (ed) Mycota IX. Springer, Germany, 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 IX, 2nd edn. Springer, Berlin, Heidelberg, pp 231–254

Varma A, Bajaj R, Agarwal A, Asthana S, Rajpal K, Das A, Prasad R, Kharkwal AC (2013a) Memoirs of 'Rootonic'—the magic fungus. Amity University Press, India

Varma A, Kost G, Oelmuller R (2013b) *Piriformospora indica*; sebacinales and their biotechnological applications. Soil biology. Springer, Berlin, Heidelberg

Venus Y, Oelmüller R (2013) *Arabidopsis* ROP1 and ROP6 influence germination time, root morphology, the formation of F-actin bundles, and symbiotic fungal interactions. Mol Plant 6:872–886

Verma S, Varma A, Rexer KH, Hassel A, Kost G, Sarbhoy A, Bisen P, Butehorn 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, Hückelhoven 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 USA 102:13386–13391

Weiss M, Selosse MA, Rexer KH, Urban A, Oberwinkler F (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108:1003–1010. https://doi.org/10.1017/S0953756204000772

Weiss M, Sykorova Z, Garnica S, Riess K, Martos F, Krause C, Oberwinkler F, Bauer R, Redecker D (2011) Sebacinales everywhere: previously overlooked ubiquitous fungal endophytes. PLoS One 6:e16793

Weiß, M, Waller F, Zuccaro A and Selosse MA (2016), Sebacinales – one thousand and one interactions with land plants. New Phytol 211:20–40. https://doi.org/10.1111/nph.13977

Wilson D (1995) Endophyte—the evolution of a term and clarification of its use and definition. Okios 73:274–276

Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T (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 20
Arbuscular Mycorrhizal Fungi: Green Approach/Technology for Sustainable Agriculture and Environment

Priyanka Srivastava, Bhawna Saxena, and Bhoopander Giri

Abstract To feed the growing population, global food production needs to be doubled by 2050. The fertilizers cost have increased several folds in the last few years, which necessitates agrarian community to be less reliable on chemicals to grow and protect their crops. Moreover, dependency on chemical fertilizers and pesticides has led to the deterioration of human health, disruption of ecosystem functioning and degradation of our environment. To overcome these problems, there is a need to explore and exploit the beneficial plant–soil microbe interactions to meet the food demand without affecting the relationship between the man and his environment. Arbuscular mycorrhizal fungi (AMF) are known to form symbiotic association with the roots of more than 90% of the terrestrial plants. They serve as biofertilizer and enhance the plant growth by accelerating nutrient uptake, particularly of inaccessible nutrients like phosphorus and nitrogen from the soil. Beside mineral nutrition, AMF also maintain the root hydraulic conductivity, increase the plant net photosynthetic capacity, improve stomatal conductance. The multifunctional extraradical hyphae of the fungus provide numerous ecological advantages like maintaining the soil health by influencing the beneficial microbes, aggregating soil particle and preventing soil erosion, conferring resistance to various stresses, enhance ecosystem productivity, bioremediation of degraded land, serving as soil carbon sink. In this chapter we attempt to discuss different role played by AMF, which make them potential tool for sustainable agriculture and environment. It is tempting to state that AMF served for 3E's i.e. eco-friendly, economic and enhanced yield.

P. Srivastava
Department of Botany, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

B. Saxena • B. Giri (✉)
Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi 110036, India
e-mail: bhoopg@yahoo.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_20

20.1 Introduction

The world's population is growing rapidly and has been predicted to reach around nine billion by the middle of this century (Rodriguez and Sanders 2015), which will put a tremendous pressure on the global agriculture to meet the growing demand for food. Therefore, the increase in crop production and land productivity in agriculture is necessary. It is beyond the doubt that due to the application of chemical fertilizers and agrochemicals, food production in agriculture has increased significantly. The use of these chemicals has been predicted to be increased significantly in future too (Weber 2014). However, the frequent use of chemical fertilizers along with other chemicals like pesticides and weedicides or herbicides has already generated environmental issues such as deterioration of soil quality, surface and ground water, soil biodiversity and ecosystem functioning. Any increase in the dosage of these chemicals to promote the agricultural productivity, however would severely deteriorate our environment and agriculture. Therefore, in order to safeguard the environment health as well as agriculture productivity, it is importance to reduce dependence of farmers on these agrochemicals to promote plant growth and yield.

Soil is an excellent habitat for a wide array of microorganisms, which could play specific role in maintaining soil productivity, ecological processes and environment health. These microbes could play a key role in sustainable agriculture as they improve the fertility and health of the soil. They safeguard plant from enemies, enhance nutrient cycling and assist host plant to acquire immobile nutrients like N and P from the plant (Aggarwal et al. 2011; Wagg et al. 2014; Bender et al. 2016; Hunter 2016a, b) also established symbiotic associations with a wide range of plant species (Rillig et al. 2016) and thereby benefit their partner plant. The application of beneficial soil microbes could be a potential source to sort-out the issue of intensive use of costly chemical fertilizers for agricultural production as they can increase nutrient availability, plant tolerance against various kinds of stresses and therefore can provide a sustainable way for agricultural practices (Aggarwal et al. 2011). In the soil, these microorganisms are present either in the free-living (Plant Growth Promoting Rhizobacteria, like *Azotobactor, Azospirillum, Pseudomonads*) state or may develop mutual association with plant roots (*Rhizobia*, mycorrhizas and mycorrhiza-like organisms like *Piriformospora indica*) (Prasad et al. 2015).

Amongst the diverse groups of soil microorganisms, mycorrhizas are the most ubiquitous soil fungi (Schüßler et al. 2001; Smith and Read 2008; Gianinazzi et al. 2010; Leifheit et al. 2014). It has been predicted that mycorrhizal fungi may have existed even when the first plants appeared on land, which is estimated around more than 400 million years ago (Brundrett 2002). They form a mutual symbiotic relationship with the roots of plant. They are called mycorrhiza originated from the Greek 'mukés', meaning fungus, and 'rhiza' meaning roots. So far, seven different mycorrhizal fungi have been discovered from natural soils, out of these arbuscular mycorrhizal fungi (AMF) are rather common amongst the wide range of plant species. More than 90% of terrestrial plant species are colonized by AMF (Gomes et al. 2017). AMF exist in two environments; in the soil, where they form

an extensive extraradical mycelium, which scavenges mineral nutrients, and within the root, where they grow between and within cortical cells developing symbiotic interfaces-the finger-like profusely branched arbuscules or intracellular coils and balloon like structures, the vesicles (Smith and Smith 2011). Arbuscules are the functional sites of nutrient transfer and vesicles act as storage organs. Extraradical hyphae extend beyond the depletion zone and support host plant for acquiring mineral nutrients and water from the soil that are not easily accessible to the normal root system. This AMF–plant relationship encourages plant growth and development of root (Kaur et al. 2014). In return, fungus avail food/carbon from the partner plant. The fungus utilizes carbon/carbohydrate for its own growth and reproduction and also in the synthesis of excretory molecules like glycoprotein (known as glomalin), which release to the soil and helps in improving soil structure with soil organic matter content (Kaur et al. 2014; Sharma et al. 2017). AMF reproduce through asexual mode of reproduction. The sexual mode of reproduction in AMF has not yet well understood. AMF are potential components of sustainable management systems. These fungi are known to exist in a wide range of environment and play a vital role in maintaining plant–water relations, nutrient uptake and ionic balance and improve soil quality and health and productivity of plants (Ruiz-Lozano et al. 2012; Nadeem et al. 2014). Under abiotic stresses, they could improve accumulation of osmo-protectants, maintain membrane integrity and osmotic adjustment and prevent oxidative damages thereby reduce adverse effects of environmental stresses and improve plant growth (Wu and Xia 2006; Evelin et al. 2009). In addition, AMF notably enhance accumulation of active ingredients in several herbal medicinal plants (Mandal et al. 2013). It is estimated that AMF could reduce up to 50% usage of chemical fertilizers for optimal agriculture production depending upon plant species and environmental conditions. Therefore, AMF could be a potential tool for sustainable agricultural practices with improved agronomic strategies which can improve fertility, health and environment of the soil, thereby the plant growth and yield (Aggarwal et al. 2011; Bender et al. 2016; Rillig et al. 2016). In this review, we attempt to highlight the role of AMF as an essential component of sustainable agriculture and environment, due to their involvement in the increased nutrient uptake, biomass production, improved photosynthesis capacity, improving quality and quantity of plant secondary metabolites, reducing dependence on fertilizers and other agro-chemical and improving plant's tolerance to environmental stresses.

20.2 Green Approaches in Relation to Agriculture and Environment

The development of agriculture sector, defined in terms of increased production with decreased average cost along with healthy and safe environment. Sustainable agriculture includes four main objectives; (i) environmental health management (including plant and soil), (ii) economic effectiveness and (iii) enhanced yield, and

(iv) social and financial justice (Brodt et al. 2011). The techniques, which make products and processes more sustainable and environmental friendly may be considered as green or clean approaches. Green approaches aim to make the environment sustainable by reducing environmental degradation, conserving natural resources and reducing emissions of green house gases (CO_2, N_2O, CH_4 etc).

20.3 Potential Solutions for Sustainable Agriculture and Environment

In the previous decades, the consumption of chemical fertilizers and pesticides has been increased massively to increase the crop production to meet the growing demands of rising population for food, which has largely affected the environment and ecosystem of the agricultural fields in the form of deterioration of soil quality and fertility, contamination of surface and ground water, air pollution and decrease in ecosystem functioning and biodiversity (Schultz et al. 1995; Socolow 1999). A potential solution to fulfill current and future generation requirements could be efficient use of the community waste and sewage sludge, which is an inexpensive alternative as manure. However, these sources are having high amount of heavy metal content, which could impose negative impact on the soil microorganisms, and heavy metals may get accumulated in the crop itself and crop field (Giller et al. 1998; Graham 2000).

Soil contains the diverse groups of microorganisms, which could play specific roles in maintaining the soil productivity and ecological processes. To overcome the environmental and ecological issues along with increased crop yield, the application of soil microorganisms could be a cheaper, affordable and eco-friendly solution (Aggarwal et al. 2011). The utilization of beneficial soil microbes for the sustainable agriculture and environment could connect sustainability with enhanced productivity, and agricultural productivity could be efficiently maintained by careful planning of conservation as well as utilization of soils (managing soil fertility/quality). Transferring/minimizing existing agro-practices (use of chemical fertilizers and pesticides) with microbial applications is not only economical to grow safe and sufficient food but also for keeping the environment clean and contamination free. Further, sustainable agriculture with organic produce is paying attention of farmers, governments and development agencies. Organic farming is found in coordination with the local environment using land husbandry techniques such as soil-conservation measures, crop rotation, and the application of agronomic, biological and manual methods instead of synthetic inputs like mineral fertilizers and agro-chemicals for maximum outputs. Organic agriculture is a cost-effective approach for economically weaker farmers who cannot pay for expensive techniques of green revolution (Manimozhi and Gayathri 2012). Since arbuscular mycorrhizal fungi colonize roots of a majority of land plant species, provide them with many benefits and play a key role in ecosystem management and functioning, their application could be a potential

approach for sustainable agricultural practices, and the improved agronomic strategies with AMF can improve the health of soil and environment (Schüßler et al. 2001; Aggarwal et al. 2011).

20.4 Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (also known as Endomycorrhizae or AMF) are soil fungi, which forms symbiotic association with roots of many vascular plants (including crop plants). AMF are obligate symbionts, belonging to the phylum Glomeromycota, which comprises many genera and species (Schüßler et al. 2001). They colonize all angiosperm families except Betulaceae, Utricaceae, Commelinaceae, Cyperaceae and Polygonaceae. AMF are present as a bridge between roots and soil, where they provide the host plant with mineral nutrients and water from the soil in exchange of carbohydrates from host plant for their own growth and reproduction (Fig. 20.1) (Smith and Read 2008). In this symbiotic

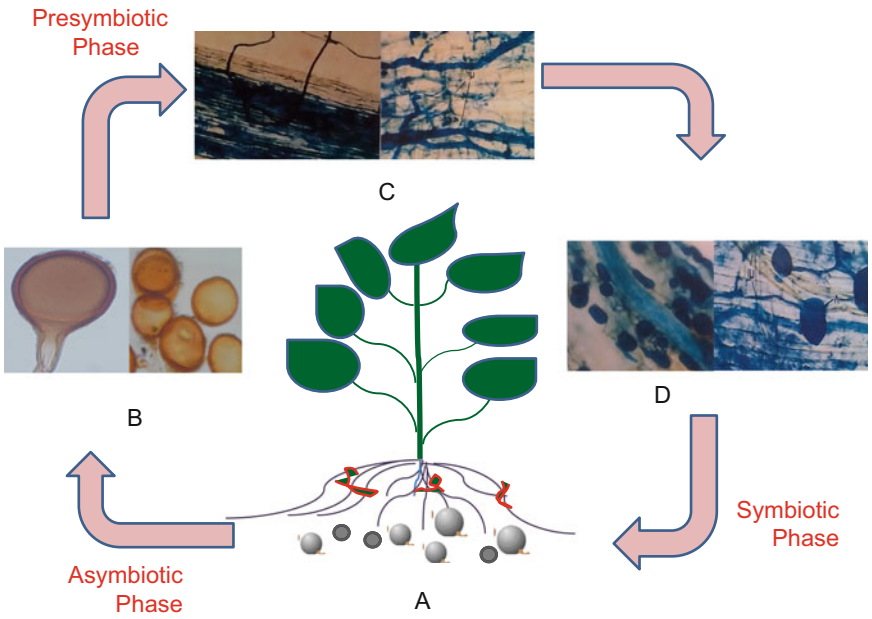


Fig. 20.1 Propagation cycle of arbuscular mycorrhizal fungi. They are considered asexual because sexual reproduction is remaining to be studied. The asexual life cycle includes three main phases, asymbiotic, presymbiotic and symbiotic phases. AM fungal spores present in the soil communicate with plant root (**A**), start germinating (**B**) subsequently develop dense extraradical hyphae, which grow through soil particles and eventually enter plant roots (**C**), forming a bulbous structure called appresorium. Inside plant root, fungal hyphae grow between and within cortical cells, forming intracellular arbuscules and intraradical vesicles (**D**)

association, the fungus develops different structures in soil and in root of host plant. They develop two types of hyphal network, the extraradical hyphae (present in soil) and intraradical hyphae (present in plant root). The extraradical hyphae extract important nutrients and forms dense branched filamentous structures in the soil (Smith and Read 2008). They extend several meters far from the root and are responsible for acquisition of mineral nutrients and water for host plant along with propagation of the fungal spore. AMF fungi produce different types of soil hyphae like thick or runner hyphae and thin or absorptive hyphae (Friese and Allen 1991). The hyphal network is long-lived, and is able to colonize new plant roots as they come in contact with.

Arbuscular mycorrhizal symbiosis initiates as the fungal hyphae start responding to the presence of a plant root, establishing a molecular communication and growing along its surface. Indeed, one or a few hyphae produce appressoria (a kind of swelling like structure), these appressoria penetrate epidermal cells of the root and proceed for entering the root (Brundrett 2008). These hyphae penetrate the hypodermis, start profusely branching in the root cortex region and spread in both directions and form a network. Where, the hyphae develop highly dichotomously branched structure called arbuscules. Arbuscules are formed between the cell wall and the cell membrane of root. They start to form about 2 days after root penetration and never come into the direct contact with the nucleus or other cell organelles of root cell (Brundrett et al. 1985). The exchange in this mutual association occurs in such a way that fungus facilitates plant to uptake nutrients from soil and plant supply carbohydrate to the fungus. This exchange of nutrients takes place at the cell membrane-arbuscule interface (Balestrini and Bonfante 2014). Indeed, AMF hyphae also produce balloon-like swellings in the root cortex known as vesicles, which accumulate the storage products. These arbuscules may be inter- or intracellular and may function as propagules by developing thick walls in older roots (Biermann and Linderman 1983; Brundrett 2008; Balestrini and Bonfante 2014). Due to the presence of vesicles and arbuscules these fungi were referred to as arbuscular mycorrhizal or AM, however, it has been observed that a few species of AMF do not produce vesicles; therefore, it was suggested to refer as arbuscular mycorrhizal fungi. AMF reproduce asexually through thick-walled spores produced on the extraradical hyphae, and stay in the soil for long periods.

20.5 Role of Arbuscular Mycorrhizal Fungi in Sustainable Agriculture

20.5.1 Plant Growth and Productivity

AMF supply amount of nutrients and water to crop plants, helping in overcoming stresses therefore lead to boost the productivity of various crops (Lekberg and Koide 2005; Suharno et al. 2017). Karagiannidis and Hadjisavva-Zinoviadi (1998)

observed twofold increase in the biomass production of *Triticum turgitum* var. durum inoculated with *Glomus mosseae*, grow in ten different soils. Improved productivity and improved content of protein, Fe and Zn was recorded in mycorrhizal chick pea (Pellegrino and Bedini 2014). In alkaline soil, the synergistic effect of AMF and *Rhizobium leguminosarum* bv. *viciae* was observed on the growth of *Vicia faba* L. (Abd-Alla et al. 2014). Some of the AMF species, such as *Glomus geosporum* and *Glomus claroideum* accumulate heavy metals of soil into plant roots and prevent their translocation to shoot, acting as a filter (Sambandan et al. 1992; Del Val et al. 1999; Leyval et al. 2002; Meier et al. 2015). They enhance productivity and quality of plant in contaminated soils. Under saline condition, wheat varieties colonized with AMF have shown increased productivity, which may be attributed to the mycorrhiza-assisted reduced uptake of Na and Cl and improved uptake of mineral nutrients by wheat plants (Daei et al. 2009).

20.5.2 Mineral Nutrition

AMF provide host plant with mineral nutrients (Smith and Read 2008). The extraradical mycelium of AMF emerged out from the host root, extends into the soil and increases the surface area to acquire locked nutrients from the soil and alleviate nutrient deficiency by providing adequate supply of mineral nutrients to host plant (Marschner and Dell 1994; Johnson et al. 2010; Nouri et al. 2014). Phosphorus (P) and nitrogen (N) both are important determinants for the AM symbiosis and the colonization of the host plant is controlled by feedback mechanisms between both nutrients (Kytoviita 2005; Fellbaum et al. 2014).

Nitrogen is the most important nutrient for plant development and AMF assist plants to avail more than 50% of required nitrogen from soil and organic compounds by the way of N cycling and producing large amount of external hyphae (Hodge and Fitter 2010; McFarland et al. 2010; Veresoglou et al. 2012a). Studies suggest that AMF can obtain substantial amount of N from decayed organic matter/residue or accelerate N mineralization from organic matter (Atul-Nayyar et al. 2009; Hodge and Fitter 2010) and also affect the carbon supply through soil microbial communities during decomposition (Herman et al. 2012). AMF improves symbiotic nitrogen fixation, nodulation and increase the activities of pectinase, xylo-glucanase and cellulose which are involved in the nitrogen metabolism and increase the decomposition of soil organic matter (Barea 1991). AMF may exert a priming effect on the soil bacterial communities shifting denitrifying communities by reducing the availability of soluble N and can also reduce denitrification and N_2O emission rates (Ames et al. 1984; Amora-Lazcano et al. 1998; Scheublin et al. 2010; Veresoglou et al. 2012b; Bender et al. 2014).

AMF-inducible NO_3^- or NH_4^+ transporters have been identified in several plants including tomato and soyabean (Hildebrandt et al. 2002; Kobae et al. 2010). The NH_4^+ ammonium transporter has been found to be expressed in arbuscules, suggesting control of AMF on the supply of nutrients to host (Kobae et al. 2010).

Comparing with typical uptake systems of plants, the $NH4^+$ uptake system of AMF have a five times higher affinity for $NH4^+$, which enables the fungus to obtain $NH4^+$ from the soil even under low N supply conditions (Pérez-Tienda et al. 2012). Another important source of N in the soil is free amino acids. AMF influence the uptake of several of these amino acids such as aspartic acid, serine, glycine, glutamic acid, glutamine, cysteine or methionine (Cliquet et al. 1997; Nakano et al. 2001). Monoxenic root-organ culture experiments have shown that translocation of N in plant occurs as arginine but converts to $NH4^+$ before transfer to the plant across the symbiotic interface (Smith and Smith 2011). In *Sorghum bicolor*, AMF increase the uptake of phenylalanine, methionine, asparagine, tryptophan, and cysteine, along with increased uptake of the charged amino acids like arginine, lysine, and histidine, which seems to be possible with the help of such transporters only (Cappellazzo et al. 2008; Whiteside et al. 2012).

P is an essential nutrient for plant but difficult to be acquired from the soil (Lambers et al. 2015) due to its extremely low diffusion rate (Shen et al. 2011). Mycorrhizal fungi release phosphatases, which solubilize soil organic P and increase the uptake of P by both responsive and non-responsive plants (Smith et al. 2003; Li et al. 2006). Further, phosphatases act on phytate, a major source of organic P in the soil, and release the H_2PO_4 which is utilized by the plant (Joner et al. 2000). Mycorrhizal fungi increase the exploration of P in soil, increase its translocation to plants through arbuscules, help in efficient transfer of P to plant roots and increased storage of absorbed P (Bhat and Kaveriappa 2007). AMF–plant interaction specifically induces the expression of plant Pi transporters (Harrison et al. 2002; Balestrini et al. 2007; Walder et al. 2015). In sorghum (*Sorghum bicolor*) and flax (*Linum usitatissimum*) two genes coding for inorganic phosphate transporters are identified that were induced in roots colonized by AMF but acquisition of inorganic phosphorus (Pi) was strongly affected by the combination of plant and AMF species (Walder et al. 2016). The utilization of AMF along with phosphate solubilising bacteria and other soil microbes could improve plant productivity even with low grade rock phosphates as a source of P (Bagyaraj et al. 2015). However, the effectiveness of acquisition of P varies between fungal species and even between isolates within a species (Smith and Read 2008). Jansa et al. (2005) reported that *Glomus mosseae* and *Glomus intraradices* are more efficient in P uptake than *Glomus claroideum* and *Glomus mosseae*.

Along with N and P, AMF also help in transfer of sulfur (S) to the plants with the help of sulfate transporters (Allen and Shachar-Hill 2009; Giovannetti et al. 2014). Plants take up S primarily as the sulfate anion, which is often found in low concentrations in the soil (Leustek 1996; Allen and Shachar-Hill 2009). Several researchers have reviewed the uptake, transfer and utilization of S by plants (Allen and Shachar-Hill 2009; Rennenberg et al. 2007). Sulfur is a mobile element. It is commonly lost through soil leaching. Therefore, a large proportion of the soil S remains present in the organically bound forms such as sulfate esters, synthesized by soil microorganisms, which is difficult to utilize by the plant (Fitzgerald 1976; Scherer 2001; Allen and Shachar-Hill 2009). Mycorrhizal fungi have been found to increase the percentage of S in pot-grown onion and maize plants under the

conditions of low S availability (Mohamed et al. 2014; Guo et al. 2007). However, very little is known about the mechanism of S assimilation and its regulation in mycorrhizal fungi. Allen and Shachar-Hill (2009) conducted research experiments to determine the role of AMF in acquisition of S, its metabolism and transfer to host roots and to identify effectors of S transfer to host roots. They found that sulfate was taken up by the AMF and transferred to AMF-colonized roots, increasing root S contents under moderate concentration of sulfate.

20.5.3 Water Uptake/Root Hydraulic Conductivity

Root hydraulic conductivity and permeability are the reliable indicators for water uptake activity in plants (Aubrecht et al. 2006). Mycorrhizal mycelium network affects moisture retention properties of soils and improves water uptake capacity of plant by regulating stomatal conductance (Augé et al. 2015). AMF inoculated plants have shown altered root morphology and hydraulic conductivity, demonstrating that increased root hydraulic conductivity is favorable for plant growth as it credits to improved water uptake by host plant (Augé 2001; Cseresnyés et al. 2013). Cseresnyés et al. (2014) observed that the mycorrhizal bean and cucumber plants exhibit higher rate of daily transpiration and root electrical capacitance than that of the non-mycorrhizal plants. Ruiz-Lozano (2003) revealed that to mitigate the water deficiency, AMF help to improve water uptake by enhancing the absorptive surface area of root (Aggarwal et al. 2011) which ultimately increase the soil water movement into root of plants and delay the decline in water potential (Porcel and Ruiz-Lozano 2004).

20.5.4 Role of AMF in Managing Rate of Photosynthesis

Mycorrhizal symbiosis increases photosynthetic capacity of plants by increasing the activity of hormones like cytokinin, gibberellins and of auxins which could elevate the rate of photosynthesis by influencing stomatal opening (Allen et al. 1982). Increasing colonization of *Boswellia papyrifera* seedlings resulted in greater stomatal conductance (Birhane et al. 2012). The inoculation of *Robinia pseudoacacia* L. (Black Locust) with AMF, *Funneliformis mosseae* and *Rhizophagus intraradices* grow in a lead toxic soil has shown increased photosynthetic pigment content in leaves and higher gas exchange capacity, non-photochemistry efficiency, and photochemistry efficiency (Yang et al. 2015). AMF greatly influence the photosynthesis of black locust seedlings with significantly greater leaf area, higher carboxylation efficiency, chlorophyll content and net photosynthetic rate. They significantly increase the photochemical efficiency of PS II and enhance the carbon content and calorific value of the seedlings (Zhu et al. 2014). Similarly, *Citrus tangerine* (tangerine) colonized by *Glomus versiforme*

exhibited higher photosynthetic rates, stomatal conductance, transpiration rate and improve osmotic adjustments, substantiating influence of AMF on plant photosynthesis (Wu and Xia 2006).

20.5.5 Accumulation of Secondary Metabolites/Active Ingredients

Plant metabolites are divided into two broad categories, primary and secondary. The primary metabolites are vital for the basic processes of life including photosynthesis and respiration whereas secondary metabolites are important defense and specialized metabolites noteworthy for the plant interaction with its environment. Secondary metabolites play significant role in defense, and protection of plants against biotic and abiotic stress. AMF studies performed on herbal and aromatic medicinal plants showed that mycorrhizal symbiosis influences plant secondary metabolism in both above and belowground parts of the plants, increasing accumulation of active ingredients (Table 20.1). Farmer et al. (2007) observed accumulation of β-carotene in the tuber of *Ipomoea batatas*. Similarly, Sailo and Bagyaraj (2005) found increasing level of diterpene forskol in the roots of *Coleus forskohlii*. On mycorrhization of *Glycyrrhiza uralensis* plants, Liu et al. (2007) recorded higher concentration of glycyrrhizin. Mandal et al. (2013) carried out research experiments to examine the influence of AMF, *Rhizophagus fasciculatus* on the yield of secondary metabolites in *Stevia rebaudiana*, in relation to mycorrhiza-induced physiological changes in addition to improved P uptake. AMF inoculation of *Stevia* with P-supplementation produced higher concentrations of steviol glycosides in comparison to control plants. The higher content of stevioside and rebaudioside-A (steviol glycosides) in AMF inoculated plants could be attributed to increased nutrients uptake and biomass production, and carbohydrates and jasmonic acid, contributing to more biosynthesis of steviol glycosides. Enhanced nutrient uptake and sugar concentration due to increased photosynthesis in AMF inoculated stevia could control up-regulation of the transcription of steviol glycosides biosynthesis genes (Mandal et al. 2015). Mycorrhizal symbiosis also facilitates the accumulation of essential oils in plants like *Anethum graveolens*, *Trachyspermum ammi* and *Foeniculum vulgare* (Kapoor et al. 2002, 2004; Mandal et al. 2013). Inoculation of *Glycyrrhiza uralensis* with *Glomus mosseae* has been found to improve accumulation of flavonoids, liquiritin, isoliquiritin, isoliquiritigenin and glycyrrhizic acid (Chen et al. 2017) under nutrient stress condition. Marjoram and lemon balm inoculation with AMF considerably increased the yield of rosmarinic acid and lithospermic acid isomers (Engel et al. 2016). Similarly, in Lettuce they enhanced the accumulation of vitamins, nutraceuticals and minerals (Baslam et al. 2013). In *Dioscorea* spp., the inoculation of *Glomus etunicatum* increased content of polyphenols, flavonoids and anthocyanin (Lu et al. 2015). These observations substantiate the fact that the quantity and quality of

Table 20.1 Influence of AMF on accumulation of active ingredients in plants

Active ingredients produced	AMF species involved	Host plant	Family	Reference
Glycyrrhizin	*Glomus mosseae*	*Glycyrrhiza uralensis*	Fabaceae	Liu et al. (2007)
Hypericin and pseudohypericin	*Rhizophagus intraradices*	*Hypericum perforatum*	Hypericaceae	Zubek et al. (2012)
Polyphenols, flavonoids, and anthocyanin	*G. etunicatum*	*Dioscorea sp*	Dioscoreaceae	Lu et al. (2015)
Camptothecin	*G. diaphanum, Acaulospora mellea and Sclerocystis sinuosa*	*Camptotheca acuminata*	Nyssaceae	Zhao et al. (2007)
Flavonoids, lignin, DPPH activity and phenolic compounds	*Funneliformis mosseae*	*Cucumis sativus* L. seedlings	Cucurbitaceae	Chen et al. (2013)
Phenol	*Glomus aggregatum*	*Catharanthus roseus*	Apocynaceae	Srinivasan and Govindasamy (2014)
Chicoric and Caffeic acid derivative	*Rhizophagus intraradices*	*Ocimum basilicum*	Lamiaceae	Scagel and Lee (2012)
Stevioside and rebaudioside	*Rhizophagus fasciculatus*	*Stevia rebaudiana*	Asteraceae	Mandal et al. (2013)
Linalool and Methyl chavicol	*Glomus sp.*	*Ocimum basilicum*	Lamiaceae	Zolfaghari et al. (2012)
2-hydroxy-4-methoxy benzaldehyde	*Glomus sp.*	*Decalepis hamiltonii*	Asclepidaceae	Matam and Parvatam (2017)
Cinnamic acid, anthocyanin, Total phenol	*Glomus etunicatum and Glomus mosseae*	*Mentha spicata*	Lamiaceae	Bagheri et al. (2014)
Total phenol	*Glomus intraradices*	*Ocimum gratissimum L*	Lamiaceae	Hazzoumi et al. (2015)
Phytoalexins	*Glomus fasciculatum*	*Vigna unguiculata*	Leguminaceae	Sundaresan et al. (1993)
Rishitin and solavetivone	*Glomus etunicatum*	*Solanum tuberosum*	Solanaceae	Yao et al. (2003)
Trigonelline	*Gigaspora rosea*	*Prosopis laevigata*	Leguminaceae	Rojas-Andrade et al. (2003)
Cyanidin-3-glucoside	*Glomus intraradices*	*Fragaria x ananassa Duch.*	Rosaceae	Castellanos-Morales et al. (2010)

(continued)

Table 20.1 (continued)

Active ingredients produced	AMF species involved	Host plant	Family	Reference
Linalool, linalyl acetate	*Acaulospora morrowiae, Rhizophagus clarus, Scutellospora calospora*	*Mentha × piperita L. var. citrata (Ehrh.) Briq.*	Lamiaceae	Silva et al. (2014)
Artemisinin, Jasmonic acid	*Rhizophagus intraradices*	*Artemisia annua*	Asteraceae	Mandal et al. (2015)
Flavonoids, Carotenoids	*Glomus intraradices*	*Bituminaria bituminosa*	Fabaceae	Pistelli et al. (2015)
p-hydroxybenzoic acid, Rutin	*Rhizophagus irregularis*	*Viola tricolor L.*	Violaceae	Zubek et al. (2015)
Ferulic acid, Caffeic acid, Kaempferol and Luteolin, Quercetin	*Piriformospora indica*	*Brassica campestris ssp. chinensis L.*	Brassicaceae	Khalid et al. (2017)
Tanshinones, Salvianolic acid B	*Glomus mosseae, Glomus aggregatum, Glomus versiforme, Glomus intraradices*	*Salvia miltiorrhiza*	Lamiaceae	Yang et al. (2017)

active ingredients of plants could be improved by the application of optimized inoculum of arbuscular mycorrhizal fungi.

20.5.6 Abiotic Environmental Stress Tolerance

Salinity stress is one of the serious threats, limiting the plant growth and productivity by decreasing water uptake, adversely affecting nutrient absorbance, hydraulic conductivity, stomatal conductance and net photosynthetic rate (Al-Karaki et al. 2001; Koca et al. 2007). Mycorrhizal associations have been found to influence the physiological and morphological properties of plant, thereby help the plant to combat biotic and abiotic stresses (Miransari et al. 2008; Evelin et al. 2009; Saxena et al. 2017; Giri and Saxena 2017). Under environmental stresses like drought and salinity they play an important role in maintaining ionic balance and nutrient supply in plant and soil for proper functioning and productivity of crop plants (Porcel et al. 2012; Evelin et al. 2013; Augé et al. 2015). Availability of water is the major

limitation in agricultural fields particularly in arid and semi-arid areas. Several studies have shown that presence of AMF in water-stressed plants increase leaf and root growth and positively maintain root to shoot ratio (Quilambo 2000). The extraradical hyphae increase the absorptive surface area of roots for the better uptake of mineral nutrients and water under stress conditions (Hampp et al. 2000). Moreover, AMF aid plant to overcoming the adverse effects of environmental stresses as they improve balance between K:Na and Ca:Na (Elhindi et al. 2017). AMF facilitate plant for the accumulation of osmo-protectants, osmotic adjustment and maintenance of membrane integrity, therefore preventing plant from oxidative damage during environmental stresses (Evelin et al. 2013). Molecular studies have shown that in AMF colonized plants like *Glycine max* and *Lactuca sativa* changes gene expression of gene encoding plasma membrane aquaporins in response to drought stress (Porcel et al. 2005). Similarly, AM symbiosis could regulate expression of genes involved in oxidative stress, proline synthesis and in dehydrin proteins (Porcel et al. 2005; Fan and Liu 2011). Several nutritional and non-nutritional mycorrhiza-mediated mechanisms, helping plants to overcome salinity stress may be programmed as (1) nutrient uptake (like P, N, Mg and Ca) (Evelin et al. 2009), (2) Biochemical changes like accumulation of proline, betaines, polyamines, carbohydrates and enzymatic and non-enzymatic antioxidants (Evelin et al. 2009), (3) Physiological changes like enhanced photosynthetic efficiency, gas exchange and water use efficiency (Ruiz-Lozano et al. 2012; Elhindi et al. 2017; Saxena et al. 2017).

20.5.7 Weed Control

In agricultural fields, weeds are a major hazard to the crops, as they compete with crops and negatively affect the crop productivity (Oerke 2006; Ampong-Nyarko and Datta 1991). Although the management of weeds is possible using herbicides, weedicides and toxic chemicals, but the drawbacks of using these techniques are (Heap 2015; Shakeel and Yaseen 2016); (i) they are expensive, (ii) not suitable for sensitive crops (iii) several weeds are resistant against the herbicides, (iv) not safe for ecosystem and environment. Arbuscular mycorrhizal fungi have been found effective against several weeds, hence could be considered as the potent microorganisms to control agricultural weeds (Rinaudo et al. 2010; Veiga et al. 2011). Lendzemo (2004) revealed that *Striga hermonthica* (witch weed), which seriously affects the cereal crops can be controlled by applying AMF inoculants, as they directly reduce growth of weed or suppress their growth by increasing the competitiveness of crop through increased nutrient uptake of crop for essential nutrients leaving weeds deprived of nutrition. Rinaudo et al. (2010) recorded about 47% reduction in biomass due to invasion of six weeds, which were grown together with a crop, which could be controlled using AMF (Veiga et al. 2011; Adeyemi et al. 2015). Another mycorrhizal approach in weed control can be inhibiting weed growth by changing the relative abundance of mycorrhiza-infected and nonmycorrhizal-infected weed species in agroecosystem (Jordan et al. 2000) most likely due to secretion of some substances from AMF extraradical mycelium (Veiga et al. 2012).

20.5.8 Disease Resistance

Through several mechanisms like damage compensation, direct competition for colonization sites or food, changes in root morphology and rhizosphere microbial community composition, biochemical changes associated with plant defense mechanisms and the activation of plant defense. AMF can induce control over various plant diseases (Huang et al. 2003; Whipps 2004). Shalaby and Hanna (1998) observed improved growth of AMF colonized Soyabean plant in a soil infested with pathogenic fungi *Fusarium solani* and other pathogens. Similarly, AMF colonization increased resistance in Poinsettia against pathogens like *Pythium ultimumon* (Kaye et al. 1984). Coffee plant infested with mycorrhizal fungi enhanced competitiveness against disease causing *Bidens pilosa* (França et al. 2016) whereas Tea plant inoculated with mycorrhizal fungi and other plant growth promoters provided resistance for rot disease (Chakraborty et al. 2016). *Fusarium* induces wilt disease in tomato worldwide, at large its control and management is difficult. Inoculation of tomato with AMF has shown control in the severity of the disease and enhanced overall growth in tomato seedlings (Mwangi et al. 2011).

20.6 Role in Ecosystem and Environment Management

20.6.1 Soil Health and Fertility

One of the most important functions of the mycorrhizal symbiosis is to enhance acquisition of nutrients from the soil with the help of fungal hyphae. The extraradical hyphae of AM fungi often acquire those nutrients, which are hard to be extracted by plant roots. Although phosphorus is an essential nutrient for plants but remain present in the immobile form in the soil. It can be solubilized with the enzymes released by AMF (Joner and Johansen 2000). Ecosystems instability severely influences the physical, chemical and biological properties of the soil. However, mycorrhizal fungi have been found to improve such properties/processes (Augé 2004). They not only indeed affect the individual plant, even actively engaged in the processes like soil aggregation at the ecosystem level and improve the soil health and quality (Rillig 2004a, b). Nonetheless, soil aggregation helps in improving soil porosity and gaseous exchange, and nicely maintains soil nutrient cycle, protects soil carbon in aggregates, and influences beneficial soil micro-fauna (Diaz-Zorita et al. 2002; Jastrow et al. 1998; Johansson et al. 2004). By acting as a biofertilizer, and biocontrol agent mycorrhiza benefits the overall soil health and productivity (Jeffries and Barea 2012; Abdel Latef et al. 2016). Although plants absorb both macro-and micronutrients from the soil to accomplish their metabolic activities, the excessive accumulation of heavy metals like Zn, Cu, Pb, As, Cd, Cu etc in soil due to various anthropogenic activities and deposition of agro-chemicals rigorously degrade the soil and its fertility and productivity (Liu et al. 1997).

Several researchers have observed that AMF could play important role in mitigating such effects by minimizing the negative impact of heavy metal contamination attributed to their enhanced adsorption and precipitation (Nadeem et al. 2014; Turnau et al. 2008; Malekzadeh et al. 2011).

20.6.2 Ecosystem Functioning and Biodiversity

The relationships between mycorrhizal fungi and plant is of great importance as they could play a major role in the ecosystem functioning and maintaining soil biodiversity (Gerz et al. 2016), contributing to biogeochemical cycles, food and timber production, and other benefits (Gianinazzi et al. 2010; van der Heijden et al. 2015; Bender et al. 2016). In fact, AMF are involved in many biogeochemical cycles, the most studied are C, N, and P (Smith and Read 2008; Prieto et al. 2012; Ekblad et al. 2013; Hodge and Storer 2015; Mayor et al. 2015; Lazcano et al. 2014). Moreover, their widespread distribution among a wide array of plant species makes them a powerful tool to manage agricultural and environment sustainability (Azul et al. 2014; Bhardwaj et al. 2014). Mycorrhizal fungi influence plant community structure and play a pivotal role in plant community assemblage and succession (Hartnett and Wilson 1999; Heneghan et al. 2008; Kikvidze et al. 2010; Lin et al. 2015). Further, AMF facilitate the regeneration and emergence of newly developed seedlings (Stanley et al. 1993; Barea et al. 2002), alter species interactions, and change the dynamics of plant communities' thereby increasing plant diversity in terrestrial ecosystems (van der Heijden et al. 1998; Klironomos et al. 2000; Dhillion and Gardsjord 2004; Simard and Austin 2010; Horn et al. 2017). AM association enhances plant's survival rate under unfavourable soil conditions, hence increasing the plant community diversity and ecosystem productivity (van der Heijden et al. 1998; Klironomos et al. 2000; Dhillion and Gardsjord 2004). It has been noticed that ecosystem instability is increasing in the modern agriculture system that might be due to lack of AMF interactions in soil (Helgason et al. 1998; Jeffries and Barea 2012). However, the incorporation of AMF could reverse the threat to biodiversity and ecosystem instability in the present day cultivation (Tilman 1996).

20.6.3 Reclamation of Degraded Land/Bioremediation

Increase in industrialization, urbanization, mining and several other human-induced activities across the globe have led to increased accumulation of toxic elements/heavy metals in the groundwater and soil (Sharma et al. 2017), which largely affect both soil fertility as well as plant productivity (Whitmore 2006). AMF play an impending role in the restoration of degraded waste lands (Asmelash et al. 2016; Nicolson 1967). The extraradical hyphae of AMF improve the vegetation cover of the degraded lands by significantly enhancing the assimilation of nutrients (Jha

et al. 1994; Khan 2006; Gohre and Paskowski 2006). AMF attribute to the seedlings emergence and their establishment and survival in the polluted areas/lands and maintain the diversity in severely heavy metals contaminated soils (Hassan et al. 2011; Karthikeyan and Krishnakumar 2012; Manaut et al. 2015). AMF alleviate the impact of heavy metals by immobilizing them in the soil, chelation upon absorption, adsorption on the cell wall, altering pH of the rhizosphere soil and by enhancing phytostabilization and phytoextraction ability of the plants (Gaur and Adholeya 2004; Malekzadeh et al. 2011; Abdel Latef et al. 2016). Garg and Singla (2012) observed that inoculation with AMF increased the chlorophyll and relative water content in pea plants growing in the arsenic contaminated soils. Shabani et al. (2016) reported reduced transportation of Ni from the root to shoot along with the enhanced level of ABC transporter and metalothein on inoculation with *Glomus mossae* and *Festuca arundinacea* growing in the Ni polluted soil. Several studies have demonstrated that molecular expression of genes like Zn (*GintZnT1*), As (*GiArsA*), Cu and Cd (*GintABC1*, *GmarMt1*) likely to play a crucial role in plant's heavy metal tolerance (Lanfranco et al. 2002; Gonzalez-Guerrero et al. 2005; Lenoir et al. 2016). AMF predominantly prevail in the stressed habitats and unusable soils, and establish a mutual relationship with the plant species growing on the toxic soil (Cabral et al. 2015; Lenoir et al. 2016). The glomalin produced by AMF adsorbs the heavy metal from the soil and reduce the soil toxicity (Gil-Cardeza et al. 2014; Wu et al. 2014a, b). Experimentation under axenic conditions has shown that AMF exhibit potential for bioremediation of contaminated soils (Mugnier and Mosse 1987; Declerck et al. 2005; Cabral et al. 2010). In the light of occurrence and importance of AMF in the degraded waste land, it may be tempting to state that AMF could prove to be a potential approach to reclaim disturbed/contaminated soils. However, to establish the fact further research, particularly at molecular level is required.

20.6.4 Preventing Soil Erosion

Increasing soil erosion due to anthropogenic activities has become a potential cause of land degradation and desertification. Besides, increasing applications of P fertilizers of which only a small amount is absorbed by the plant and rest of it gets washed-off due to the tillage-induced soil erosion and reduced water retention capacity of soil. Since AM symbiosis plays key role in ecosystem functioning, these fungi could be applied to overcoming the problem of soil erosion (Miller and Jastrow 1990; Perumal and Maun 1999; Enkhtuya et al. 2003; Estaun et al. 2007). Of all the processes involved, production of mucilage, glomalin related soil protein (GRSP) and other extracellular compounds are the most studied one. These compounds provide strength to soil particles to formulate aggregations (Wright and Upadhaya 1996; Rillig et al. 2002; Rillig 2004a, b). A positive correlation has been found between the length of AMF hyphae and water stable aggregate through the production of glomalin related soil protein (GRSP) (Miller

and Jastrow 1990; Wright and Anderson 2000; Rillig et al. 2002). Glomalin related soil protein has also been found to maintain stability of soil. Mycorrhiza-induced GRSP fraction has a major role in aggregate stability in citrus rhizosphere (Wu et al. 2014a, b). However, soil aggregate stability may differ among the AMF species. Schreiner et al. (1997) observed a variation in the size of the aggregate formed by *Glomus mossae,* which were larger than those formed by *G. etunicatum* and *Gigaspora rosae*. Miller and Jastrow (1990) revealed a direct correlation between the spore density and water stable aggregate. Beside glomalin production, AMF-mediated dense hyphae production, entanglement of soil particles with hyphae, improved soil microbiota also play important role in soil aggregation (Rillig et al. 2002; Rillig and Mummey 2006; Sharma et al. 2017). Thus, in order to avail maximum benefits of AMF for a soil improvement program more studied are required to better understand, how AMF get involved in the formation of soil aggregation and what are other mechanisms behind it must be examined?

20.6.5 Mitigation of Global Warming and Climate Change

In addition to promoting plant growth, AMF can directly or indirectly contribute to the stabilization of carbon in the soil. AMF extraradical mycelium (AEM) has a carbon-rich component known as glycoprotein or glomalin that can stay in the soil for decades. AMF seem to have a priming impact on the other soil microbes to convert plant waste into stable soil carbon (Cheng et al. 2012), attributing to reduce atmospheric carbon-based green house gases (Giri and Saxena 2017). Research experiments conducted to study climate change impact have assessed that there is already too much GHGs in the atmosphere. To stop the progress of and reverse the effects of human-induced climate change, GHGs production must be reduced and controlled in the atmosphere. Plants have in built capacity, if harnessed appropriately, to fix carbon back into the soil in sufficient amount to make a significant contribution to combat climate change. AMF facilitate supply of the mineral nutrients to the host plant indeed increase the growth and biomass of the plant, harnessing more atmospheric carbon. Further, the sugar produced by the host is a prerequisite of AMF to meet their C requirement for own growth and life-cycle (Giri and Saxena 2017). AMF could play a vital role in the global carbon cycle, because these fungi can exploit a large percentage of the carbon fixed by their host (about 20%) under ambient atmospheric CO_2 conditions (Jakobsen and Rosendahl 1990, Drigo et al. 2010). They could help in depositing slow cycling organic compounds and protect organic matter from microbial attack (Smith and Read 2008; Verbruggen et al. 2013). This exhibits that AMF in fact promote aggregation of soil particles and help in managing soil erosion (Wilson et al. 2009). AMF help in soil C sequestration, particularly under elevated CO_2 concentration (Treseder 2016) as these fungi are the only producers of glomalin (a recalcitrant glue-like glycoprotein), which enters into the soil on the death of fungal mycelium and become a source of soil organic carbon sink (Wright et al. 1996, Wright and Upadhaya 1996).

In addition to atmospheric CO_2, nitrous oxide (N_2O) is another effective greenhouse gas involved in the destruction of the protective ozone layer in the stratosphere. It contributes to global warming with a several times higher global warming potential than atmospheric CO_2 (Forster et al. 2007; Ravishankara et al. 2009). Montzka et al. (2011) demonstrated that after carbon dioxide and methane, the nitrous oxide place highest impact on the greenhouse effect. The importance of N_2O is likely to increase due to its prolonged existence and a predicted increase in future emissions. However, the ecological processes regulating N_2O emissions from soil are not well known. Bender et al. (2014) revealed that the presence of AMF, which have a profound impact on a wide range of ecosystem functions, could shrink N_2O emissions from soil. They manipulated the abundance of AMF in two independent greenhouse experiments and two different soils and found that N_2O emissions increase by 42 and 33% in microcosms with decreased AMF abundance than microcosms with well-established AMF propagules. These results suggested that the N_2O emission from soil is controlled by AMF. They further explained that the reduced N_2O emission in the atmosphere could partially be attributed to the increased N immobilization into microbial or plant biomass and decreased level of soil N as a substrate for N_2O emission and altered water relations. Bender and his colleagues (2014) concluded that the intensification of agricultural practices may further contribute to increased N_2O emissions as it disrupts the development and proliferation of AMF hyphal network. At the high soil moisture levels AMF control N_2O emissions by increasing use of soil water (Lazcano et al. 2014).

20.7 Research Need and Approaches

AMF characteristics make them a potential tool to be utilized for the sustainable management of agriculture and environment (Rodriguez and Sanders 2015; Bhardwaj et al. 2014). Nevertheless, several agronomic practices including crop rotation, soil tillage, use of fertilizers and pesticides largely influence abundance and infectivity of mycorrhizal fungi (Jansa et al. 2002). These practices disturb AMF hyphal networks, destruct their colonization of roots and decrease the absorption of phosphorus from the soil (Douds et al. 1995). Further, conventional tillage significantly decreases mycorrhizal diversity (Alguacil et al. 2008) whereas in zero tillage condition AMF sporulation increased twofold, even in highly fertilized soil (Brito et al. 2011; Verzeaux et al. 2017). Further, land and air pollution, mining, deforestation, and many biotic and abiotic factors largely impacts mycorrhizal survivability, and cause severe loss to the viability of mycorrhizal propagules, resulting in a significant reduction in the mycorrhizal colonization of roots (Gehring and Bennett 2009; Zobel and Öpik 2014; Antoninka et al. 2015; Borriello et al. 2015; Klabi et al. 2015). Therefore, it important to understand that; (1) better understanding of the relative contribution of AMF to any aspect to sustainability by attaining a broad view of all the possible pathways by which they can influences sustainability, including their interactions with other soil microbial biome (Rillig

et al. 2016), (2) Focus on the conservation and management of AMF diversity and abundance to accomplish the goal of sustainability. Special attention is required towards the sensitive ecosystems, which are directly affected due to inadequate soil management and climate change such as glaciers (Haeberli and Beninston 1998; Jiang and Zhang 2015), and highly exploited ecosystems like mountains (Kohler and Maselli 2009; Gurung and Bajracharya 2012) should also be prioritised for conservation.

Green technology/approach not only includes the decrease in use of chemical fertilizers but also the enhancement of the sustainable techniques. For the exploitation of AMF as a tool for green technology, we aim to optimize its benefits by increasing its abundance and diversity (Rillig et al. 2016) in the soil through various strategies including (1) Field study Assessment, (2) Analysis, (3) Management and Protection policies, (4) Advanced Improvement techniques, (5) Field trials with AMF (Fig. 20.2). In field study, various soil parameters like physical (structure), chemical (pH, moisture, etc), nutrient status and abundance and diversity of beneficial microorganisms (mycorrhiza and associated microbes) are monitored, as the abundance and diversity of AMF are influenced by the soil chemistry (Casazza et al.

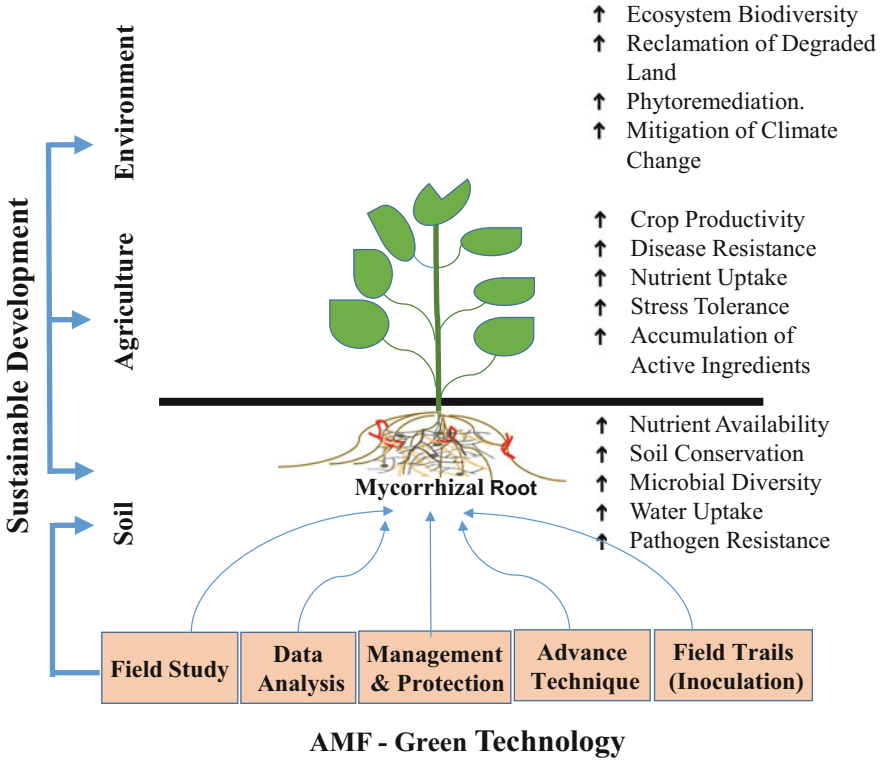


Fig. 20.2 An overview of various approaches associated with AMF-colonized plant roots, and their possible influencing role in sustainable agriculture and environment

2017). Data from field assessment records show the status of soil of the studied field and about the existing mycorrhizal and other microbial community which further provide site-specific information requirement about management and protection of AMF abundance and functioning (Rillig et al. 2016). Various crop management practices have shown improved yield in relation to mycorrhizal abundance (Monreal et al. 2015). Strategies with zero or less tillage and cover cropping have also shown enhanced root colonization and density of AMF in agriculture fields (Brito et al. 2013; Bowles et al. 2016). Moreover, advanced technologies of microbial community engineering and techniques like plant breeding are required to maintain the abundance and diversity of beneficial mycorrhiza and for manipulation and improvement of AMF with desirable traits respectively (Mueller and Sachs 2015; Hohmann and Messmer 2017). Production and field applications of AMF inoculum is the direct way to enhance the AMF propagules density in the agricultural field (Solaiman et al. 2014; Hijri 2016); however, constrain is the functioning of AMF in collaboration with many other microbes.

20.8 Conclusion and Future Perspectives

To enhance the ecosystem functioning and agricultural productivity without disturbing the balance of ecosystems and environment, utilization of arbuscular mycorrhizal fungi as a biofertilizer could be a potential solution; however, to achieve increased production, a major constraint is the development of AMF culture, abundance and density in the crop fields. Although AMF play a positive role in maintaining soil and plant health, the re-establishment of natural level of AMF richness is a major task, which could substitute the harmful chemical fertilizers and recompense a way to sustainable agriculture and environment. AMF could be utilized as a sustainable tool to improve the concentration of both macro- and micronutrients; hence could be an alternative to agronomic bio-fortification. Since AMF facilitate plants with macro- and micronutrients, it could positively influence the herbage, yield and quality of the crop, which is probably difficult to achieve using agro-chemicals. Therefore, we can recommend that field inoculation with AMF, depending upon the types of plant species and their environmental conditions, could be an effective alternative to agrochemicals. As the population is increasing day-by-day, there is a need of increased food production too, which could be only possible with the help of sustainable techniques or green techniques, like AMF-based bio-fertilizations along with PGPRs. To begin with, field trials with AMF inoculants and farmer's awareness towards cost-effective techniques are important to reducing input of agrochemicals in the agriculture. Once agricultural fields are enriched with microbial inoculants, this approach can be of great significant for managing sustainable agriculture and environment at the large scale.

References

Abd-Alla MH, El Enany AWE, Nafady NA, Khalof DM, Morsy FM (2014) Symbiotic interaction of *Rhizobium leguminosarum* bv viciae and Arbuscular mycorrhizal fungi as plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soils. Microbiol Res 169:49–58

Abdel Latef AA, Hashem A, Rasool A, Abd_Allah EF, Alqarawi AA, Dilfuza E, Sumira Jan, Naser AA, Parvaiz A (2016) Arbuscular mycorrhizal symbiosis and abiotic stress in plants: a review. J Plant Physiol 59:407–426

Adeyemi OR, Atayese MO, Dare MO, Sakariyawo SO, Adigbo SO, Bakare TO (2015) Weed control efficacy and arbuscular mycorrhizal (AM) colonization of upland rice varieties as affected by population densities. J Biol Agric Healthcare 5:178–185

Aggarwal A, Kadian N, Tanwar A, Yadav A, Gupta KK (2011) Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. J Appl Nat Sci 3:340–351

Alguacil MM, Lumini E, Roldan A, Salinas-García JR, Bonfante P, Bianciotto V (2008) The impact of tillage practices on arbuscular mycorrhizal fungal diversity in subtropical crops. Ecol Appl 18:527–536

Al-Karaki GN, Hammad R, Rusan M (2001) Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. Mycorrhiza 11:41–47

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Allen MF, Moore TS, Christensen M (1982) Phytohormone changes in *Bouteloua gracilis* infected by vesicular–arbuscular mycorrhizae. II. Altered levels of gibberellin-like substances and abscisic acid in the host plant. Can J Bot 60:468–471

Ames RN, Reid CPP, Ingham ER (1984) Rhizosphere bacterial population responses to root colonization by a vesicular arbuscular mycorrhizal fungus. New Phytol 96:555–563

Amora-Lazcano E, Vazquez MM, Azcon R (1998) Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. Biol Fertil Soils 27:65–70

Ampong-Nyarko K, Datta SK (1991) A handbook for weed control in rice. International Rice Research Institute, Manila, Philippines, p 113. ISBN-13:9789712200205

Antoninka AJ, Ritchie ME, Johnson NC (2015) The hidden Serengeti-Mycorrhizal fungi respond to environmental gradients. Pedobiologia (Jena) 58:165–176

Asmelash F, Bekele T, Birhane E (2016) The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. Front Microbiol 7:1095. https://doi.org/10.3389/fmicb.2016.01095

Atul-Nayyar A, Hamel C, Hanson K, Germida J (2009) The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. Mycorrhiza 19:239–246

Aubrecht L, Staněk Z, Koller J (2006) Electrical measurement of the absorption surfaces of tree roots by the earth impedance methods: 1. Theory. Tree Physiol 26:1105–1112

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Augé RM, Toler HD, Saxton AM (2015) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25:13–24

Azul AM, Nunes J, Ferreira I, Coelho AS, Veríssimo P, Trovão J, Campos A, Castro P, Freitas H (2014) Valuing native ectomycorrhizal fungi as a Mediterranean forestry component for sustainable and innovative solutions 1. Botany 92:161–171

Bagheri S, Ebrahimi MA, Davazdahemami S, Moghadam JM (2014) Terpenoids and phenolic compounds production of mint genotypes in response to mycorrhizal bioelicitors. TJEAS J 4:339–348

Bagyaraj DJ, Sharma MP, Maiti D (2015) Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. Curr Sci 108:1288–1293

Balestrini R, Bonfante P (2014) Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. Front Plant Sci 5:237. https://doi.org/10.3389/fpls.2014.00237

Balestrini R, Goìmez-Ariza J, Lanfranco L, Bonfante P (2007) Laser microdissection reveals that transcripts for five plants and one fungal phosphate transporter genes are contemporaneously present in arbusculated cells. Mol Plant Microbe Interact 20:1055–1062

Barea JM (1991) Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. Adv Soil Sci 15:1–40

Barea JM, Gryndler M, Lemananceau P, Schuepp H, Azcon R (2002) The rhizosphere of mycorrhizal plants. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture: from genes to bioproducts. Birkhauser, Basel

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. Agriculture 3:188–209

Bender SF, Plantenga F, Neftel A, Jocher M, Oberholzer H-R, Koehl L, Giles M, Daniell TJ, van der Heijden MGA (2014) Symbiotic relationships between soil fungi and plants reduce N_2O emissions from soil. ISME J 8:1336–1345

Bender SF, Wagg C, van der Heijden MGA (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trends Ecol Evol 31:440–452

Bhardwaj D, Ansari M, Sahoo R, 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. https://doi.org/10.1186/1475-2859-13-66

Bhat PR, Kaveriappa KM (2007) Effect of AM fungi on the growth and nutrition uptake in some endemic Myristicaceae members of the Western ghats, India. In: Tiwari M, Sati SC (eds) The mycorrhizae: diversity, ecology and application. Daya Pub. House, Delhi, pp 295–309

Biermann B, Linderman RG (1983) Increased geranium growth using pre-transplant inoculation with a mycorrhizal fungus. J Am Soc Hortic Sci 108:972–976

Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904

Borriello R, Berruti A, Lumini E, Beffa MTD, Scariot V, Bianciotto V (2015) Edaphic factors trigger diverse AM fungal communities associated to exotic camellias in closely located Lake Maggiore (Italy) sites. Mycorrhiza 25:253–265

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR (2016) Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. J Appl Ecol. https://doi.org/10.1111/1365-2664.12815

Brito I, Carvalho M, Goss MJ (2011) The importance of no-till in the development of cropping systems to maximize benefits of arbuscular mycorrhiza symbiosis. In: Elizabeth Stockdale E, Watson C (eds) Proceedings of the Association of applied biologist "Making crop rotations fit for the future". Aspects of applied biology, pp 137–141

Brito I, Carvalho M, Goss MJ (2013) Soil and weed management for enhancing arbuscular mycorrhiza colonization of wheat. Soil Use Manag 29:540–546

Brodt S, Six J, Feenstra G, Ingels C, Campbell D (2011) Sustainable agriculture. Nat Edu Know 3:1

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Brundrett MC (2008) Mycorrhizal associations: the web resource. Date accessed

Brundrett MC, Piche Y, Peterson RL (1985) A developmental study of the early stages in vesicular arbuscular mycorrhiza formation. Can J Bot 63:184–194

Cabral L, Siqueira J, Soares C et al (2010) Retention of heavy metals by arbuscular mycorrhizal fungi mycelium. Quím Nova 33:25–29

Cabral L, Soares CR, Giachini AJ, Siqueira JO (2015) Arbuscular mycorrhizal fungi in phytoremediation of contaminated areas by trace elements: mechanisms and major benefits of their applications. World J Microbiol Biotechnol 31:1655–1664

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

Casazza G, Lumini E, Ercole E, Dovana F, Guerrina M, Arnulfo A, Minuto L, Fusconi A, Mucciarelli M (2017) The abundance and diversity of arbuscular mycorrhizal fungi are linked to the soil chemistry of screes and to slope in the Alpic paleo-endemic *Berardia subacaulis*. PLoS One 12(2):e0171866. https://doi.org/10.1371/journal.pone.0171866

Castellanos-Morales V, Villegas J, Wendelin S, Vierheilig H, Eder R, Cardenas-Navarro R (2010) Root colonization by the arbuscular mycorrhizal fungus *Glomus intraradices* alters the quality of strawberry fruits (*Fragaria x ananassa* Duch.) at different nitrogen levels. J Sci Food Agric 90:1774–1782

Chakraborty K, Bose J, Shabala L, Shabala S (2016) Difference in root K^+ retention ability and reduced sensitivity of K^+ permeable channels to reactive oxygen species confer differential salt tolerance in three *Brassica* species. J Exp Bot 67:4611–4625

Chen SC, Jin WJ, Liu AR, Zhang SJ, Liu DL, He CX (2013) Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. Sci Hortic (Amst) 160:222–229

Chen M, Yang G, Sheng Y, Li P, Qui H, Zhou X, Huang L, Chao Z (2017) *Glomus mosseae* inoculation improves the root system architecture, photosynthetic efficiency and flavonoids accumulation of Liquorice under nutrient stress. Front Plant Sci 8:931. https://doi.org/10.3389/fpls.2017.00931

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Ruffy TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087

Cliquet JB, Murray PJ, Boucaud J (1997) Effect of the arbuscular mycorrhizal fungus *Glomus fasciculatum* on the uptake of amino nitrogen by *Lolium perenne*. New Phytol 137:345–349

Cseresnyés I, Takács T, Végh RK, Anton A, Rajkai K (2013) Electrical impedance and capacitance method: a new approach for detection of functional aspects of arbuscular mycorrhizal colonization in maize. Eur J Soil Biol 54:25–31

Cseresnyés I, Takács T, Füzy A, Rajkai K (2014) Simultaneous monitoring of electrical capacitance and water uptake activity of plant root system. Int Agrophys 28:537–541

Daei G, Ardakani M, Rejali F, Teimuri S, Miransari M (2009) Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. J Plant Physiol 166:617–625

Declerck S, Strullu D, Fortin J (2005) In vitro culture of mycorrhizas. Springer, New York

Del Val C, Barea JM, Azcón-Aguilar C (1999) Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. Appl Soil Ecol 11:261–269

Dhillion SS, Gardsjord TL (2004) Arbuscular mycorrhizas influence plant diversity, productivity, and nutrients in boreal grasslands. Can J Bot 82:104–114

Diaz-Zorita M, Perfect E, Grove JH (2002) Disruptive methods for assessing soil structure. Soil Tillage Res 64:3–22

Douds DD Jr, Galvez L, Janke RR, Wagoner P (1995) Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric Ecosyst Environ 52:111–118

Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO_2. Proc Natl Acad Sci USA 107:10939–10942

Ekblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjøller R et al (2013) The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant Soil 366:1–27

Elhindi KM, El-Din AS, Elgorban AM (2017) The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). Saudi J Biol Sci 24(1):170–179

Engel R, Szabó K, Abrankó L, Rendes K, Füzy A, Takács T (2016) Effect of arbuscular mycorrhizal fungi on the growth and polyphenol profile of marjoram, lemon balm, and marigold. J Agric Food Chem 64:3733–3742

Enkhtuya B, Oskarsson U, Dodd JC, Vosatka M (2003) Inoculation of grass and tree seedlings used for reclaiming eroded areas in Iceland with mycorrhizal fungi. Folia Geobot 38:209–222

Estaun V, Vicente S, Calvet C, Camprubi A, Busquets M (2007) Integration of arbuscularmycorrhiza inoculation in hydroseeding technology. Effects on plant growth and interspecies competition. Land Degrad Dev 18:621–630

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 (2013) Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum–graecum*. Mycorrhiza 23:71–86

Fan Q, Liu J (2011) Colonization with arbuscular mycorrhizal fungus affects growth, drought tolerance and expression of stress-responsive genes in *Poncirus trifoliata*. Acta Physiol Plant 33:1533–1542

Farmer MJ, Li X, Feng G, Zhao B, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V, Van Tuinen D (2007) Molecular monitoring of field-inoculated AMF to evaluate persistence in sweet potato crops in China. Appl Soil Ecol 35:599–609

Fellbaum CR, Mensah J, Cloos A, Pfeffer P, Strahan G, Kiers ET, Bücking H (2014) Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. New Phytol 2:646–656

Fitzgerald JW (1976) Sulfate ester formation and hydrolysis: potentially important yet often ignored aspect of sulfur cycle of aerobic soils. Bacteriol Rev 40:698–721

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey DW (2007) Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB et al (eds) Climate change: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York, NY

França AC, de Freitas AF, dos Santos EA, Grazziotti PH, de Andrade Júnior VC (2016) Mycorrhizal fungi increase coffee plants competitiveness against *Bidens pilosa* interference. Pesqui Agropecu Trop Goiânia 46:132–139

Friese CF, Allen MF (1991) The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. Mycologia 83:409–418

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 86:528–534

Gehring C, Bennett A (2009) Mycorrhizal fungal-plant-insect interactions: the importance of community approach. Environ Entomol 38:93–102

Gerz M, Bueno CG, Zobel M, Moora M (2016) Plant community mycorrhization in temperate forests and grasslands: relations with edaphic properties and plant diversity. J Veg Sci 27:89–99

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

Gil-Cardeza ML, Ferri A, Cornejo P et al (2014) Distribution of chromium species in a Cr-polluted soil: presence of Cr (III) in glomalin related protein fraction. Sci Total Environ 493:828–833

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agriculture soils: a review. Soil Biol Biochem 30:1389–1414

Giovannetti M, Tolosano M, Volpe V, Kopriva S, Bonfante P (2014) Identification and functional characterization of a sulfate transporter induced by both sulfur starvation and mycorrhiza formation in *Lotus japonicus*. New Phytol 204:609–619

Giri B, Saxena B (2017) Response of arbuscular mycorrhizal fungi to global climate change and their role in terrestrial ecosystem C and N cycling. In: Varma A, Prasad R, Tuteja N (eds) Mycorrhiza-functions, diversity and state of the art. Springer, Cham, pp 305–327

Gohre V, Paskowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122

Gomes SIF, Merckx VSFT, Saavedra S (2017) Fungal-host diversity among mycoheterotrophic plants increases proportionally to their fungal-host overlap. Ecol Evol 10:3623–3630

Gonzalez-Guerrero M, Azcon-Aguilar C, Mooney M (2005) Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol 42:130–140

Graham JH (2000) Assessing cost of arbuscular mycorrhizal symbiosis in agrosystems. In: Podila GK, Donds DD (eds) Current advances in mycorrhizae research. APS Press, St Paul, pp 127–140

Guo T, Zhang JL, Christie P, Li XL (2007) Pungency of spring onion as affected by inoculation with arbuscular mycorrhizal fungi and sulfur supply. J Plant Nutr 30:1023–1034

Gurung J, Bajracharya RM (2012) Climate change and glacial retreat in the Himalaya: implications for soil and plant development. Kathm Univ J Sci Engin Tech 8:153–163

Haeberli W, Beninston M (1998) Climate change and its impacts on glaciers and permafrost in the Alps. AMBIO J Hum Environ 27:258–265

Hampp R, Nehls U, Wallenda T (2000) Physiology of mycorrhiza. In: Esser K, Kadereit JW, Lüttge U, Runge M (eds) Progress in botany. Genetics, physiology, systemates, ecology. Springer, Berlin, pp 223–254

Harrison MJ, Dewbre GR, Liu JY (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413–2429

Hartnett DC, Wilson WT (1999) Mycorrhizae influence plant community structure and diversity in tall grass prairie. Ecology 80:1187–1195

Hassan SE, Boon E, St-Arnaud M, Hijri M (2011) Molecular biodiversity of arbuscular mycorrhizal fungi in trace metal-polluted soils. Mol Ecol 20:3469–3483

Hazzoumi Z, Moustakime Y, Elharchli EH, Khalid AJ (2015) Effect of arbuscular mycorrhizal fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (*Ocimum gratissimum* L). Chem Biol Technol Agric 2:10. https://doi.org/10.1186/s40538-015-0035-3

Heap I (2015) The international survey of herbicide resistant weeds. Retrieved from www.weedscience.org

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431

Heneghan L, Miller SP, Baer S, Callaham MA, Montgomery J, Pavao-Zuckerman M (2008) Integrating soil ecological knowledge into restoration management. Restor Ecol 16:608–617

Herman DJ, Firestone MK, Nuccio E, Hodge A (2012) Interactions between an arbuscular mycorrhizal fungus and a soil microbial community mediating litter decomposition. FEMS Microbiol Ecol 80:236–247

Hijri M (2016) Analysis of a large dataset form field mycorrhizal inoculation trials on potato showed highly significant increase in yield. Mycorrhiza 26:209–214

Hildebrandt U, Janetta K, Bothe H (2002) Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Appl Environ Microbiol 68:1919–1924

Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci USA 107:13754–13759

Hodge A, Storer K (2015) Arbuscular mycorrhiza and nitrogen: Implications for individual plants through to ecosystems. Plant Soil 386:1–19

Hohmann P, Messmer MM (2017) Breeding for mycorrhizal symbiosis: focus on disease resistance. Euphytica 213:113

Horn S, Hempel S, Verbruggen E, Rillig MC, Caruso T (2017) Linking the community structure of arbuscular mycorrhizal fungi and plants: a story of interdependence? ISME J 11:1400–1411

Huang Z, Krishnamurthy S, Panda A, Samal SK (2003) Newcastle disease virus V protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. J Virol 77:8676–8685

Hunter P (2016a) Plant microbiomes and sustainable agriculture. EMBO Rep 17:1696–1699

Hunter P (2016b) Deciphering the plant microbiome and its role in nutrient supply and plant immunity has great potential to reduce the use of fertilizers and biocides in agriculture. Sci Soc 17:1696–1699

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, Mozafar A, Anken T, Ruh R, Sanders IR, Frossard E (2002) Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza 12:225–234

Jansa J, Mozafar A, Frossard E (2005) Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. Plant Soil 276:163–176

Jastrow JD, Miller RM, Lussenhop J (1998) Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol Biochem 30:905–916

Jeffries P, Barea JM (2012) Arbuscularmycorrhiza – a key component of sustainable plant-soil ecosystems. In: Hock B (ed) The mycota, Fungal associations, vol IX, 2nd edn. Springer, Berlin, Heidelberg, pp 51–75

Jha DK, Sharma GD, Mishra RR (1994) Ecology of vesicular-arbuscular mycorrhiza. In: Prasad AB, Bilgrami RS (eds) Microbes and environments. Narendra Publishing House, Delhi, pp 199–208

Jiang C, Zhang L (2015) Climate change and its impact on the eco-environment of the three-rivers headwater region on the Tibetan Plateau, China. Int J Environ Res Public Health 12:12057–12081

Johansson RC, Gowda PH, Mulla DJ, Dalzell BJ (2004) Metamodelling phosphorus best management practices for policy use: a frontier approach. Agric Econ 30:63–74

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RA (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci USA 107:2093–2098

Joner EJ, Johansen A (2000) Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi. Mycol Res 104:81–86

Joner EJ, Briones R, Leyval C (2000) Metal-binding capacity of arbuscular mycorrhizal mycelium. Plant Soil 226:227–234

Jordan NR, Zhang J, Huerd S (2000) Arbuscular-mycorrhizal fungi: potential roles in weed management. Weed Res 40:397–410

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 93:3007–3011

Karagiannidis N, Hadjisavva-Zinoviadi S (1998) The mycorrhizal fungus *Glomus mosseae* enhances growth, yield and chemical composition of a durum wheat variety in 10 different soils. Nutr Cycl Agroecosyst 52:1–7

Karthikeyan A, Krishnakumar N (2012) Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Sm. seedlings inoculated with arbuscular mycorrhizal fungi. Ann For Res 55:207–216

Kaur R, Singh A, Kang JS (2014) Influence of different types mycorrhizal fungi on crop productivity. Curr Agric Res 2:51–54

Kaye JW, Pfleger FL, Stewart EL (1984) Interaction of *Glomus fasciculatum* and-*Pythium ultimum* on greenhouse-grown poinsettia. Can J Bot 62:1575–1579

Khalid M, Hassani D, Bilal M, Liao J, Huang D (2017) Elevation of secondary metabolites synthesis in *Brassica campestris* ssp. *chinensis* L. via exogenous inoculation of *Piriformospora indica* with appropriate fertilizer. PLoS One 12(5):e0177185

Khan AG (2006) Mycorrhiza remediation-an enhanced form of phytoremediation. J Zhejiang Univ Sci B 7:503–514

Kikvidze Z, Armas C, Fukuda K, Martínez-García LB, Miyata M, Oda-Tanaka A (2010) The role of arbuscular mycorrhizae in primary succession: differences and similarities across habitats. Web Ecol 10:50–57

Klabi R, Bell TH, Hamel C, Iwaasa A, Schellenberg M, Raies A, St-Arnaud M (2015) Plant assemblage composition and soil P concentration differentially affect communities of AM and total fungi in a semi-arid grassland. FEMS Microbiol Ecol 91:1–13

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecol Lett 3:137–141

Kobae Y, Tamura Y, Takai S, Banba M, Hata S (2010) Localized expression of arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51:1411–1415

Koca H, Bor M, Özdemir F, Türkan İ (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot 60:344–351

Kohler T, Maselli D (2009) Mountains and climate change – from understanding to action. Published by Geographica Bernensia with the support of the Swiss Agency for Development and Cooperation (SDC), and an International Team of Contributors, Bern

Kytoviita MM (2005) Role of nutrient level and defoliation on symbiotic function: experimental evidence by tracing C-14/N-15 exchange in mycorrhizal birch seedlings. Mycorrhiza 15:65–70

Lambers H, Martinoia E, Renton M (2015) Plant adaptations to severely phosphorus-impoverished soils. Curr Opin Plant Biol 25:23–31

Lanfranco L, Bolchi A, Ros EC, Ottonello S, Bonfante P (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

Lazcano C, Barrios-Masias FH, Jackson LE (2014) Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. Soil Biol Biochem 74:184–192

Leifheit EF, Veresoglou SD, Lehmann A, Morris EK, Rillig MC (2014) Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation – a meta-analysis. Plant Soil 374:523–537

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204

Lendzemo VW (2004) The tripartite interaction between sorghum, *Striga hermonthica*, and arbuscular mycorrhizal fungi. PhD thesis, Wageningen University, Wageningen, The Netherlands

Lenoir I, Fontaine J, Lounès-Hadj A (2016) Arbuscular mycorrhizal fungal responses to abiotic stresses: a review. Phytochemistry 123:4–15

Leustek T (1996) Molecular genetics of sulfate assimilation in plants. Physiol Plant 97:411–419

Leyval C, Joner EJ, del Val C, Haselwandter K (2002) Potential of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. Birkhäuser, Basel, pp 175–186

Li HY, Smith SE, Holloway RE, Zhu YG, Smith FA (2006) Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytol 172:536–543

Lin G, McCormack ML, Guo D (2015) Arbuscular mycorrhizal fungal effects on plant competition and community structure. J Ecol 103:1224–1232

Liu C, Muchhal US, Raghothama KG (1997) Differential expression of TPSI1, a phosphate starvation-inducible gene in tomato. Plant Mol Biol 33:867–874

Liu JN, Wu LJ, Wei SG, Xiao X, Su CX, Jiang P, Song ZB, Wang T, Yu ZL (2007) Effects of arbuscular mycorrhizal fungi on the growth, nutrient uptake and glycyrrhizin production of licorice (*Glycyrrhiza uralensis* Fisch). Plant Growth Regul 52:29–39

Lu F, Lee C, Wang C (2015) The influence of arbuscular mycorrhizal fungi inoculation on yam (*Dioscorea* spp.) tuber weights and secondary metabolite content. Peer J 3:e1266

Malekzadeh E, Alikhani AH, Savaghebi-Fioozabadi RG, Zarei M (2011) Influence of arbuscular mycorrhizal fungi and an improving growth bacterium on Cd uptake and maize growth in Cd-polluted soils. Spanish J Agric Res 9:1213–1223

Manaut N, Sanguin H, Ouahmane L, Bressan M, Thioulouse J, Baudoin E (2015) Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. Ecol Eng 79:113–119

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

Manimozhi K, Gayathri D (2012) Eco friendly approaches for sustainable agriculture. J Environ Res Dev 7:166–173

Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159:89–102

Matam P, Parvatam G (2017) Arbuscular mycorrhizal fungi promote enhanced growth, tuberous roots yield and root specific flavor 2-hydroxy-4-methoxy benzaldehyde content of *Decalepis hamiltonii* Wight and Arn. Acta Sci Pol Hortorum Cultus 16:3–10

Mayor J, Bahram M, Henkel T, Buegger F, Pritsch K, Tedersoo L (2015) Ectomycorrhizal impacts on plant nitrogen nutrition: emerging isotopic patterns, latitudinal variation and hidden mechanisms. Ecol Lett 18:96–107

McFarland J, Ruess R, Keilland K, Pregitzer K, Hendrick R, Allen M (2010) Cross-ecosystem comparisons of in situ plant uptake of amino acid-N and NH4+. Ecosystems 13:177–193

Meier S, Cornejo P, Cartes P, Borie F, Medina J, Azcón R (2015) Interactive effect between Cu-adapted arbuscular mycorrhizal fungi and biotreated agrowaste residue to improve the nutritional status of *Oenothera picensis* growing in Cu-polluted soils. J Plant Nutr Soil Sci 178:126–135

Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol Biochem 22:579–584

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2008) Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biol Biochem 40:1197–1206

Mohamed AA, Wedad EEE, Heggo AM, Hassan EA (2014) Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur-oxidising bacteria on onion (*Allium cepa* L.) and maize (*Zea mays* L.) grown in sandy soil under greenhouse conditions. Ann Agric Sci 59:109–118

Monreal CM, DeRosa M, Mallubhotla SC, Bindraban PS, Dimkpa C (2015) The application of nanotechnology for micronutrients in soil-plant systems. VFRC report 2015/3. Virtual Fertilizer Research Center, Washington, DC, p 44

Montzka SA, Dlugokencky EJ, Butler JH (2011) Non-CO2 greenhouse gases and climate change. Nature 476:43–50

Mueller UG, Sachs JL (2015) Engineering microbiomes to improve plant and animal health. Trends Microbiol 23:606–617

Mugnier J, Mosse B (1987) Vesicular-arbuscular mycorrhizal infection in transformed root-inducing T-DNA roots grown axenically. Phytopathology 77:1045–1050

Mwangi MW, Monda EO, Okoth SA, Jefwa JM (2011) Inoculation of tomato seedlings with *Trichoderma Harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. Braz J Microbiol 42:508–513

Nadeem SM, Ahmad M, Zahir ZA (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environment. Biotechnol Adv 32:429–448

Nakano A, Takahashi K, Koide RT, Kimura M (2001) Determination of the nitrogen source for arbuscular mycorrhizal fungi by ^{15}N application to soil and plants. Mycorrhiza 10:267–273

Nicolson TH (1967) Vesicular-arbuscular mycorrhiza-a universal plant symbiosis. Sci Prog Oxf 55:561–581

Nouri E, Breuillin-Sessoms F, Feller U, Reinhardt D (2014) Phosphorus and nitrogen regulate arbuscular mycorrhizal symbiosis in *Petunia hybrida*. PLoS One 9:e90841

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43

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

Pérez-Tienda J, Valderas A, Camañes G, García-Agustín P, Ferrol N (2012) Kinetics of NH_4+ uptake by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. Mycorrhiza 22:485–491

Perumal JV, Maun MA (1999) The role of mycorrhizal fungi in growth enhancement of dune plants following burial in sand. Funct Ecol 13:560–566

Pistelli LA, Ulivieri V, D'Angiolillo F, Giovannelli S, Pistelli LU, Giovannetti M (2015) Influence of arbuscular mycorrhizal fungi (AMF) in the production of secondary metabolites of *Bituminaria bituminosa* L. In: Proceedings of the joint congress SIBV-SIGA, Milano, Italy

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, Gómez M, Kaldenhoff R, Ruiz-Lozano JM (2005) Impairment of NtAQP1 gene expression in tobacco plants does not affect root colonization pattern by arbuscular mycorrhizal fungi but decreases their symbiotic efficiency under drought. Mycorrhiza 15:417–423

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, 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 (PGPR) and Medicinal Plants. Springer, Cham, pp 247–260

Prieto I, Armas C, Pugnaire FI (2012) Water release through plant roots: new insights into its consequences at the plant and ecosystem level. New Phytol 193:830–841

Quilambo OA (2000). Functioning of peanut (*Arachis hypogaea* L.) under nutrient deficiency and drought stress in relation to symbiotic associations. PhD thesis, University of Groningen, The Netherlands, Van Denderen B.V., Groningen

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326:123–125

Rennenberg H, Herschbach C, Haberer K, Kopriva S (2007) Sulfur metabolism in plants: are trees different? Plant Biol 9:620–637

Rillig MC (2004a) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol Lett 7:740–754

Rillig MC (2004b) Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J Soil Sci 84:355–363

Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

Rillig MC, Wright SF, Eviner V (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238:325–333

Rillig MC, Sosa-Hernandez MA, Roy J, Aguilar-Trigueros CA, Valyi K, Lehmann A (2016) Towards an integrated mycorrhizal technology: harnessing mycorrhiza for sustainable intensification in agriculture. Front Plant Sci 7:1625

Rinaudo V, Bàrberi P, Giovannetti M, van der Heijden MGA (2010) Mutualistic fungi suppress aggressive agricultural weeds. Plant Soil 333:7–20

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

Rojas-Andrade R, Cerda-Garcia-Rojas CM, Frias-Hernandez JT, Dendooven L, Olalde-Portugal-V, Ramos-Valdivia AC (2003) Changes in the concentration of trigonelline in a semi-arid leguminous plant (*Prosopis laevigata*) induced by an arbuscular mycorrhizal fungus during the presymbiotic phase. Mycorrhiza 13:49–52

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13:309–317

Ruiz-Lozano JM, Porcel R, Azcón C, Aroca R (2012) Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. J Exp Bot 63(11):4033–4044

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

Sambandan K, Kannan K, Raman N (1992) Distribution of vesicular-arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil-Nadu, India. J Environ Biol 13:159–167

Saxena B, Shukla K, Giri B (2017) Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In: Wu QS (ed) Arbuscular mycorrhizas and stress tolerance of plants. Springer, Singapore, pp 67–97

Scagel CF, Lee J (2012) Phenolic composition of basil plants is differentially altered by plant nutrient status and inoculation with mycorrhizal fungi. Hortic Sci 47:660–671

Scherer HW (2001) Sulphur in crop production. Eur J Agron 14:81–111

Scheublin TR, Sanders IR, Keel C, van der Meer JR (2010) Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. ISME J 4:752–763

Schreiner RP, Mihara KL, McDaniel H, Bethlenfalvay GJ (1997) Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188:199–209

Schultz RC, Colletti JP, Isenhart TM, Simkins WW, Mize CW, Thompson ML (1995) Design and placement of a multi-species riparian buffer strip system. Agrofor Syst 29:1–16

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Shabani L, Sabzalian MR, Mostafavi S (2016) Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in *Festuca arundinacea*. Mycorrhiza 26:67–76

Shakeel M, Yaseen T (2016) A review on exploring the weed suppressing characteristics of arbuscular mycorrhizal fungi for enhanced plant yield and productivity. Sci Technol Dev 35:54–62

Shalaby AM, Hanna MM (1998) Preliminary studies on interactions between VA mycorrhizal fungus *Glomus mosseae*, *Bradyrhizobium japonicum* and *Pseudomonas syringae* in soybean plants. Acta Microbiol Pol 47:385–391

Sharma S, Anand G, Singh N, Kapoor R (2017) Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. Front Plant Sci 8:906. https://doi.org/10.3389/fpls.2017.00906

Sharma S, Prasad R, Varma A, Sharma AK (2017) Glycoprotein associated with *Funneliformis coronatum, Gigaspora margarita* and *Acaulospora scrobiculata* suppress the plant pathogens in vitro. Asian J Plant Pathol. https://doi.org/10.3923/ajppaj.2017

Shen J, Yuan L, Zhang J, Li H, Bai Z, Chen X (2011) Phosphorus dynamics: from soil to plant. Plant Physiol 156:997–1005

Silva VC, Alves PAC, de Oliveira RA, de Jesus RM, do Bomfim Costa LC, Gross E (2014) Influence of arbuscular mycorrhizal fungi on growth, mineral composition and production of essential oil in *Mentha* × *piperita* L. var. *citrata* (Ehrh.) Briq. under two phosphorus levels. J Med Plants Res 8:1321–1332

Simard SW, Austin ME (2010) The role of mycorrhizas in forest soil stability with climate change. In: Simard S (ed) Climate change and variability. In Tech, Rijeka, Croatia, pp 275–302

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. 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, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20

Socolow RH (1999) Nitrogen management and the future of food: lessons from the management of energy and carbon. Proc Natl Acad Sci USA 96:6001–6008

Solaiman ZM, Abbott LK, Varma A (eds) (2014) Mycorrhizal fungi: use in sustainable agriculture and land restoration. Springer, Berlin

Srinivasan R, Govindasamy C (2014) Influence of native arbuscular mycorrhizal fungi on growth, nutrition and phytochemical constituents of *Catharanthus roseus* (L.) G. Don. J Coast Life Med 2:31–37

Stanley MR, Koide RT, Shumway DL (1993) Mycorrhizal symbiosis increases growth, reproduction and recruitment of *Abutilon theophrasti* Medic. in the field. Oecologia 94:30–35

Suharno, Soetarto ES, Sancayaningsih RP, Kasiamdari RS (2017) Association of arbuscular mycorrhizal fungi (AMF) with *Brachiaria precumbens* (Poaceae) in tailing and its potential to increase the growth of maize (*Zea mays*). Biodiversitas 18:433–441

Sundaresan P, Raja NU, Gunasekaran P (1993) Induction and accumulation of phytoalexins in cowpea roots infected with the mycorrhizal fungus *Glomus fasciculatum* and their resistance to *Fusarium* wilt disease. J Biosci 18:291–301

Tilman D (1996) Biodiversity: population versus ecosystem stability. Ecology 77:350–363

Treseder KK (2016) Model behavior of arbuscular mycorrhizal fungi: predicting soil carbon dynamics under climate change. Botany 94:417–423

Turnau K, Anielska T, Ryszka P, Gawronski S, Ostachowicz B, Jurkiewicz A (2008) Establishment of arbuscular mycorrhizal plants originating from xerothermic grasslands on heavy metal rich industrial wastes – new solution for waste revegetation. Plant Soil 305:267–280

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf Engel R, Boller T, 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

Veiga RSL, Jansa J, Frossard E, van der Heijden MGA (2011) Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? PLoS One 6:e27825

Veiga RSL, Howard K, van der Heijden MGA (2012) No evidence for allelopathic effects of arbuscular mycorrhizal fungi on the non-host plant *Stellaria media*. Plant Soil 360:319–331

Verbruggen E, Veresoglou SD, Anderson IC, Caruso T, Hammer EC, Kohler J (2013) Arbuscular mycorrhizal fungi – short-term liability but long-term benefits for soil carbon storage? New Phytol 197:366–368

Veresoglou SD, Chen BD, Rillig MC (2012a) Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol Biochem 46:53–62

Veresoglou SD, Shaw LJ, Hooker JE, Sen R (2012b) Arbuscular mycorrhizal modulation of diazotrophic and denitrifying microbial communities in the (mycor)rhizosphere of *Plantago lanceolata*. Soil Biol Biochem 53:78–81

Verzeaux J, Nivelle E, Roger D, Hirel B, Dubois F, Tetu T (2017) Spore density of arbuscular mycorrhizal fungi is fostered by six years of a no-till system and is correlated with environmental parameters in a silty loam soil. Agronomy 7(2):38

Wagg C, Bender SF, Widmer F, van der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci USA 111:5266–5270

Walder F, Brulé D, Koegel S, Wiemken A, Boller T, Courty PE (2015) Plant phosphorus acquisition in a common mycorrhizal network: regulation of phosphate transporter genes of the Pht1 family in sorghum and flax. New Phytol 205:1632–1645

Walder F, Boller T, Wiemken A, Courty PE (2016) Regulation of plants' phosphate uptake in common mycorrhizal networks: role of intraradical fungal phosphate transporters. Plant Signal Behav 11:e1131372

Weber JG (2014) A decade of natural gas development: the makings of a resource curse? Resour Energy Econ 37:168–183

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227
Whiteside MD, Garcia MO, Treseder KK (2012) Amino acid uptake in arbuscular mycorrhizal plants. PLoS One 7:e47643
Whitmore A (2006) The emperors new clothes: sustainable mining? J Clean Prod 14:309–314
Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461
Wright SF, Anderson RL (2000) Aggregate stability and glomalin in alternative crop rotations for the central great plains. Biol Fertil Soils 31:249–253
Wright SF, Upadhaya A (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Plant Soil 198:97–107
Wright SF, Franke-Snyder M, Morton JB, Upadhyaya A (1996) Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181:193–203
Wu QS, Xia RX (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425
Wu Q-S, Cao M-Q, Zou YH, He XH (2014a) Direct and indirect effects of glomalin, mycorrhizal hyphae, and roots on aggregate stability in rhizosphere of trifoliate orange. Sci Rep 4:5823. https://doi.org/10.1038/srep05823
Wu Z, McGrouther K, Huang J, Wu P, Wu W, Wang H (2014b) Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: field experiment. Soil Biol Biochem 68:283–290
Yang YR, Han XZ, Liang Y, Amit G, Chen J, Tang M (2015) The combined effects of arbuscular mycorrhizal fungi (AMF) and lead (Pb) stress on Pb accumulation, plant growth parameters, photosynthesis, and antioxidant enzymes in *Robinia pseudoacacia* L. PLoS One 10(12): e0145726. https://doi.org/10.1371/journal.pone.0145726
Yang Y, Ou X, Yang G, Xia Y, Chen M, Guo L, Liu D (2017) Arbuscular mycorrhizal fungi regulate the growth and phyto-active compound of *Salvia miltiorrhiza* seedlings. Appl Sci 7:68
Yao MK, Desilets H, Charles MT, Boulanger R, Tweddell RJ (2003) Effect of mycorrhization on the accumulation of rishitin and solavetivone in potato plantlets challenged with *Rhizoctonia solani*. Mycorrhiza 13:333–336
Zhao X, Wang Y, Yan XF (2007) Effect of arbuscular mycorrhiza fungi and phosphorus on camptothecin content in *Camptotheca acuminata* seedlings. Allelopath J 20:51–60
Zhu XQ, Wang CY, Chen H (2014) Effects of arbuscular mycorrhizal fungi on photosynthesis, carbon content, and calorific value of black locust seedlings. Photosynthetica 52:247–252
Zobel M, Öpik M (2014) Plant and arbuscular mycorrhizal fungal (AMF) communities-which drives which? J Veg Sci 25:1133–1140
Zolfaghari M, Nazeri V, Sefidkon F, Rejali F (2012) Effect of arbuscular mycorrhizal fungi on plant growth and essential oil content and composition of *Ocimum basilicum* L. Iranian. J Plant Physiol 3:643–650
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 21
Rhizosphere *Mycorrhizae* Communities an Input for Organic Agriculture

M. Nayeem Sofi, Rouf Ahmad Bhat, Asmat Rashid, Naseer A. Mir, Shafat A. Mir, and Rafiq Lone

Abstract *Mycorrhizae* are an important biotic factor that influences tropical ecological succession and differently affect the woody species belonging to different successional stages. They are key components of the soil microbiota that play an essential role in plant growth, plant protection and soil quality. These fungi are widespread in agriculture systems and are especially relevant for organic farming because they can act as natural biofertilizer and enhance plant yield. The interaction between organic practices and *Mycorrhizae* populations are limited and inconsistent. Here, we explore the various roles they play in organic farming systems with special emphasis on their contribution to crop productivity. Present proceedings highlights that organic low-input systems have a high potential to maintain the *Mycorrhizae*, keeping the soil fertile and productive and point the need to incorporate AM technology in organic farming to stop deterioration of agricultural and forest land and other adverse factors. Symbiotic associations between *Mycorrhizae* and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance of heavy metals and better soil structure. However, many agricultural practices including use of fertilizers and biocides, tillage, monocultures and the growing of non-mycorrhizal crops are detrimental to *Mycorrhizae*. As a result, agro-ecosystems are impoverished in *Mycorrhizae* and may not provide the full range of benefits to the crop without *Mycorrhizae*.

M.N. Sofi
Division of Soil Science, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir 190025, India

R.A. Bhat • A. Rashid • S.A. Mir
Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir 190025, India

N.A. Mir
Faculty of Forestry, Sher-e-Kashmir University of Agricultural Sciences and Technology-Shalimar, Srinagar, Kashmir 191201, India

R. Lone (✉)
Department of Botany, SBBS University, Khiala, Jalandhar, Punjab 144030, India
e-mail: rafiqlone@gmail.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_21

21.1 Introduction

The dawn of every day poses tough challenges to farming community, producing more food to feed the burgeoning population from shrinking land and less water without eroding the ecological foundation is probably the most uphill task faced by scientists, farmers as well as policy makers. Green Revolution of 1960s gifted the Indian agriculture with fertilizer responsive high yielding varieties, high analysis fertilizers, fast acting pesticides and boosted the production of our nation to greater heights with increased cropping intensity. The use of organic manures as sources of nutrients declined sharply. But miserably in the recent past, the declining trends in productivity are more spectacular in Indian Agriculture. Chemical fertilizers are regularly applied to get maximum yields. But as a result of the chemical reactions these get fixed in the soil resulting only part of it being available over the crop period, necessitating fresh additions. The practice of dependence on inorganic fertilizers is not sustainable because these are produced in ways which can not be continued indefinitely as the resources used in their production are non renewable. Further chemical phosphatic fertilizer production is highly energy intensive process. The excessive use of these fertilizers has deteriorated the soil health and adversely affected its biodiversity. In addition the presence of heavy metals in inorganic fertilizers is well established (Chuck 2008). Eventually these heavy metals can build up to unacceptable levels especially in the vegetable produce. Average annual intake of uranium by adult is estimated to be 0.5 mg from ingestion of food and water and 0.6 μg from the breathing air. Polonium-210 contained in phosphatic fertilizers is also absorbed by the plants and stored in its tissues. This element has been found to cause about 11,700 lung cancer deaths each year world over Scholten and Timmermans (1992). Radioactive Polonium (^{210}PO) is one of the most dangerous carcinogen found in Tobacco leaves. It can be removed from tobacco by various method but risk of cancer will not decrease because there are other carcinogen other than polonium present in tobacco which can cause cancer (Chaudhari 2015). The conventional agricultural practices have caused soil erosion, reduction in water availability, increased salinization, pollution due to fertilizers, herbicides, reduced socio-economic values, degrading effect on the environment, danger to food security, quality and safety, reduced bio-diversity, lack of sustainable agricultural policies for the future generations etc., such concerns and problems posed by modern day agriculture gave birth to organic farming. Organic farming is a system which avoids or largely excludes use of synthetic inputs (fertilizers, pesticides, growth hormones, feed additives etc.) and to the maximum extent feasibly rely upon crop rotation, crop residues, animal manures, off farm organic wastes, mineral grade rock additives and biological system of nutrient mobilization and plant protection.

Organic farming is a holistic production management system which promotes and enhances agro-ecosystem health including biodiversity, biological cycles and soil biological activity, and this is accomplished by using on farm agronomic, biological and mechanical methods in exclusion of all synthetic off farm inputs.

For nutrient management under low input production system many biological inputs are used to support the plant nutrition. Among these mycorrhizae is possessing a unique position by supporting the plant growth and development through its multifaceted role. The establishment of functional symbiosis is achieved with in 4–6 weeks and thereafter the host plants derive nutritional and biological benefits from AM symbiosis.

Soil provides an ecological niche for many of the microorganisms including arbuscular mycorrhizal fungi. German botanist Albert Frank in 1885 introduced the Greek word mycorrhiza which literally means "fungus root". These fungi form the beneficial symbiotic association with the roots of higher plants and perform the function of root hair. This symbiotic association has been reported to promote plant growth and health by playing the role of biofertilizer and bioprotectant, respectively. Arbuscular mycorrhizal association is found in 80% of the plant species except *Cruciferae, Chenopodiceae, Caryophyllaceae* and *Cyperaceae* (Hirrel et al. 1978). The association also occurs over a broad ecological range from aquatic to desert environment. The mycorrhizal symbiosis has been recognized to play a key role in nutrient cycling in the eco system and to protect plants against environmental and biological stresses. In fact, many high value ornamental and edible crops enter in to some form of mycorrhizal association. Most of the crop plants are mycotrophic (i.e. they have the ability to respond to AMF symbiosis), hence functionally, AMF may benefit the productivity and/or vigour of many crops. In addition the mycorrhizal plants have greater tolerance to toxic heavy metals, root pathogens, drought, high soil temperatures, soil salinity, and adverse soil pH and to transplantation shock. Because of their wide spread occurrence in nature and their numerous benefits to plants the fungi are currently attracting much attention in agricultural, horticultural, forestry research. Though there are different mycorrhizal associations, the most common type occurring in all ecological situations, is the vesicular arbuscular mycorrhiza (Bagyaraj 1989; Barea and Jeffries 1995). Increased plant growth because of AM colonization is well documented (Bagyaraj and Varma 1995). The increased plant growth is attributed to enhanced uptake of diffusion limited nutrients, hormone production, biological nitrogen fixation, drought resistance and suppression of root pathogens. Biological control can be defined as the directed, accurate management common components of ecosystems to protect plants against pathogens. Several workers have reported that AM fungi can act as biocontrol agents for alleviating the severity of disease caused by root pathogenic fungi, bacteria and nematodes.

It is evident that an increased capacity for nutrient acquisition resulting from mycorrhizal association could help the resulting stronger plants to resist stress. However, AM symbiosis may also improve plant health through a more specific increase in protection (improved resistance and or tolerance against biotic and abiotic stresses). Mycorrhizae appear to be extremely advantageous to crops growth in low fertility soils which are characteristic of poorly managed, continuously cropped agricultural lands as well as drastically disturbed landscape and mined reclamation sites. Increases in mineral uptake as the result of mycorrhizal associations are often reflected in increased plant survival, growth and yield as well as

nutrition. The improved plant growth is attributed to increased nutrient uptake, especially phosphate, due to the exploration by the external hyphae of the soil beyond the root hair zone. The phosphate uptake is more significant in soils deficient in phosphorus. The increased growth of plants inoculated with AM fungi is not only attributed to improved phosphate uptake but also to better availability of other elements like Zn, Cu, K, S, Al, Mn, Mg, Fe etc. Allen et al. (1982) illustrated that AM affects directly the levels of plant hormones like cytokinins and gibberellin substances. Plants colonized by AM fungi can tolerate a wide range of soil water regimes ad also improve water relations of many plants. Biofertilizer help in increasing crop productivity by way of increased biological nitrogen fixation, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action orantibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out, and it can be helped achieving sustainability of farms (Khosro and Yousef 2012).

Mycorrhiza are the rule in nature, not the exception. In a mycorrhizal association, the fungus may colonize the roots of a host plant, either intracellularly or extracellularly. Mycorrhizae are present in 92% of plant families (80% of species) (Wang and Qiu 2006), with endomycorrhizae or Arbuscular Mycorrhizae (AM) being the ancestral and predominant form and indeed the most prevalent symbiotic association found in all the plant kingdom. AM are formed only by fungi in the division Glomeromycota. AM fungi lives in association with approximately 85% of herbaceous plants and produce microscopic arbuscules within cells of the root. Symbiotic associations between AM fungi and plant roots are widespread in the natural environment and can prove range of benefits to the host plant.

AM fungi play an important role in plant health by improving nutrient (especially inorganic P) and water uptake by their host plant and providing protection against soil-borne pathogens (Kurle and Pfleger 1994; Siddiqui and Mahmood 1996; Ryan and Graham 2002). In return, the fungi receive carbohydrates (sugars) and growth factors from the host plant. Other benefits include: increased resistance to foliar-feeding insects (Gange and West 1994), improved drought resistance (Auge et al. 1994) and increased tolerance of salinity and heavy metals. Increased uptake of macronutrients other than P, including N, K and Mg has also been measured as well as increased uptake of some micronutrients maintaining soil aggregate stability. Many agricultural practices including use of fertilizers and biocides, tillage, monocultures and the growing of non-mycorrhizal crops are detrimental to AM fungus communities (Kabir et al. 1998; Thingstrup et al. 1998). As a result, agroecosystems are impoverished in AMF and may not provide the full range of benefits to the crop. In natural environments, the diversity of AM fungi is a key contributor to the diversity and productivity of plant communities (Van der Heijden et al. 1998). AM fungi are strongly affected by anthropogenic activities (Giovannetti and Gianinazzi-Pearson 1994). A variety of agricultural

practices are known to impact on AMF, with fertilizers, cultivation, crop monocultures and non-mycorrhizal crop plants known to reduce inoculum (Kurle and Pfleger 1994; Helgason et al. 1998; Daniell et al. 2001).

Organic farming is the only sustainable farming system that is legally defined. It is a crop production system that avoids the use of synthetic and chemical inputs like fertilizers, pesticides, growth regulators and live stock feed additives. Indiscriminate use of synthetic chemicals and the problems arising from them forced us to think about the alternative means. To the maximum extent feasible, organic farming systems rely on the management of soil organic matter to enhance the chemical, biological and physiological properties of the soil, in order to optimize crop production. Soil management controls the supply of nutrients to crops and subsequently to live-stock and humans (Watson et al. 2002). Organic manures such as farmyard manure, compost, vermicompost, biofertilizers, biopesticides etc. can be used at least as complement, if not a substitute.

Organic systems have longer-term solutions at the systems level. An example of this system is the importance of crop rotation design for nutrient cycling and conservation and weed, pest and disease control (Stockdale et al. 2001). Organic fertilizer sources were shown to have major positive effects on the physical properties of soil. This effect is due to the role of mycorrhiza on soil structure formation (Celik et al. 2004). Soil microbial communities are considered a vital factor for the functioning of agroecosystems and success in organic farming (Gosling et al. 2006). Organic farming systems utilize highly complex and integrated biological systems to achieve their goal and most, if not all, management practices used in this system affect more than one component of the system, for example, cultivation may be beneficial for weed control but may stimulate mineralization of nitrogen when the crop does not require it. Thus, the interaction between soil management practices and different aspects of production and environmental impact will continue to challenge the nature and development of organic farming in the nature.

21.2 Interactions

21.2.1 AMF Interaction with Soil and Crops

Mycorrhizal root systems increase the absorptive area of roots 10–1000 times thereby greatly improving the ability of the plants to utilize the soil resource. AM fungi are able to absorb and transfer all of the 15 major, macro and micro nutrients necessary for plant growth (Lester 2009). This behaviour is particularly evident with soil nutrients that are more immobile such as P, Zn and Cu. The fungal soil network is able to maintain P transport to plant for longer periods (Hodge 2000; Jeffries and Barrea 1994; Lange and Vlek 2000).

Mycorrhizal fungi release powerful chemicals into the soil that dissolve hard to capture nutrients such as P, Fe and other tightly bound soil nutrients (Lester 2009).

This extraction process is particularly important in plant nutrition. AM fungi forms an intricate web that captures and assimilates nutrients conserving the nutrient capital in soils. The same extensive network of fungal filaments important to nutrient uptake are also important in water uptake and storage.

21.2.2 AMF Interaction with Agricultural Practices

Crop management involves a range of practices which have impact on the AM association, both directly, by damaging or killing AMF and indirectly, by creating conditions either favourable or unfavourable to AM fungi. In general, agricultural practices have a negative impact on the AM association and agricultural soils are AMF impoverished, particularly in terms of number of species (Helgason et al. 1998; Menendez et al. 2001). For example, high levels of P fertilization have been found to slow down or inhibit mycorrhizal efficiency in soybean fields (Ezawa et al. 2000). Higher soil infectivity was observed under reduced or no tillage practices (Mozafar et al. 2000) and limited increased mycorrhizal colonization of barley root and soil infectivity (Hamel et al. 1996). Relative to conventional management, there is evidence that organic farming practices can enhance the amounts of AMF inoculum (Bending et al. 2004; Mader et al. 2000).

21.2.3 AMF Interaction with Other Soil Micro-organisms

As well as interacting with disease causing agents, AM fungi also interact with a whole range of causal organisms in soils. AM fungi might provide a means of biocontrol of plant disease in organic systems (Siddiqui et al. 1998; Harrier and Watson 2004; Whipps 2004). Bacterial communities and some strains promote germination of AM fungal spores which will increase the rate and extent of root colonization (Johansson et al. 2004). These interactions suggest that AM might affect plant and soil microbial activity by stimulating the production of root exudates, phytoalexins and phenolic compounds (Morandi 1996; Norman and Hooker 2000).

21.3 Role of AMF Colonization on Plant Nutrition and Growth

The relationship between the development of arbuscular mycorrhizas and increased growth of the host was recognized by Asai (1944) in his studies of AM colonization and nodulation in a large number of legumes. He concluded that colonization was important both in plant growth and in the development of nodules. The C economy

of AM plants needs to be considered in the context of the effects of AM colonization on mineral nutrition and the relative costs of fungal C use, in relation to benefits derived from increased nutrient uptake.

AMF plants have two potential pathways of nutrient uptake, directly from the soil or via an AM fungal symbiont. The AM pathway depends on three essential processes: uptake of the nutrients by the fungal mycelium in the soil; translocation for some distance within the hyphae to the intraradical fungal structures (hyphae, arbuscules and coils) within the roots and transfer to the plant cells across the complex interface between the symbionts. The fungal mycelium in soil can absorb nutrients beyond the zone depleted through uptake by the roots themselves, so that they increase the effectiveness with which the soil volume is exploited. Consequently, the effects of AM colonization on P nutrition are often large and may have indirect effects on other aspects of plant metabolism, so that direct effect of the symbiosis on the other nutrients are masked (Smith and Read 2008).

21.4 AM Fungal Carbon Metabolism

AM fungi are completely dependent on an organic C supply from a photosynthetic partner. Between 4 and 20% of net photosynthate is transferred to the fungus and used in production of both vegetative and reproductive structures and in respiration to support growth and maintenance, including nutrient uptake (Smith and Read 2008).

Carbon is deployed in growth of the intra and extra radical mycelium and in respiration to support both growth and maintenance, representing a considerable increase in C flux to the soil. At this stage, there is little indications of the reasons for the variations in the estimates, but they are likely to include species of plant and fungus, fungal biomass and rate of colonization, as well as the metabolic activity of the fungus.

21.5 AM Fungi in Organic Farming Systems

AMF are potential contributors to plant nutrition and pathogen suppression in low input agricultural systems, although individual species of AMF vary widely in their functional attributes. Organic farming has developed from a wide number of disparate movements across the world into a more uniform group of farming systems, which operate broadly within the principles of the International Federation of Organic Agricultural Movements (Stockdale et al. 2001). Though the exact production methods vary considerably, general principles include the exclusion of most synthetic biocides and fertilizers, the management of soils through addition of organic materials and use of crop rotation (IFOAM 1998). The use of readily soluble fertilizers and biocides are severely restricted in organic farming. As a

result, organic systems often have lower concentrations of total and available soil P than equivalent conventional systems (Gosling and Shepherd 2005). Biocontrol agents that may used in organic systems to control pathogenic fungi do not appear to damage the AMF association (Ravnskov et al. 2002; Gaur et al. 2004).

In organic plant production, the supply of phosphorus is a bottle neck as P is the only macronutrient that cannot be obtained through biological fixation or weathering of parent rock minerals. Farmers thus rely upon recycling of nutrients from plant residues and manure, or addition of superphosphate to meet plant's demand for phosphorus. The mycorrhizal fungi form hyphae in soil as the extension of the roots, transporting nutrients from the soil to the plant. Regarding organic nutrients, mycorrhiza has been shown to improve the utility of both N and P in plant material, as its wide distribution makes more frequent contact with sites where organic matter is mineralized. This scavenging of the soil is the main mechanism for plant supply of P and some other plant nutrients in agroecosystems where these are not a soluble salts (Joner 1996).

It is widely acknowledged that AM technology can improve soil and crop productivity by allowing farmers to produce their inputs of chemical fertilizers and/or by enhancing plant survival, thus offsetting ecological and environmental concerns. Mycorrhizal fungi have particular value for legumes because of their need for an adequate phosphorus supply, not only for optimum growth but also for nodulation and nitrogen fixation (Azcon-Aguilar et al. 1979; Hayman 1986).

Both improved nitrogen fixation in legumes by *Rhizobium* and increased uptake of phosphorus from AM fungal associations can indirectly reduce the chemical fertilizer requirement and the problems related to water and air pollution by chemicals as residuals to the soil-root zone. The reduction of fertilizer requirements by using efficient isolates of *Rhizobium* and AM fungi with different leguminous agricultural crops grown in Bangladesh is of great value (Mridha and Xu 2001).

Some of the agronomically important trees, which have AM fungi association include citrus, tea, coffee, rubber and oil-palm where these plants are grown in nurseries, AMF inoculation may greatly facilitate establishment and early growth after transplanting to the field site. Nursery production of ornamental seedlings and cuttings by treating the rooting and growing media with appropriate inocula is another important area where AM can be used.

21.6 Does Organic Farming Favour AM Fungi?

Recent studies have indicated that one important contributor to plant productivity in low input systems, Mycorrhiza (AMF) have very low inoculums in conventional management systems (Mader et al. 2000). In organic farming, a package of actions is applied such as the use of crop rotation, inter and intra cropping and manuring. The soil fertility was enhanced by organic farming and the healthy crops were produced more efficiently with respect to energy and nutrient use. It was found that organically managed soil had greater AMF spore numbers and root colonization

potential and therefore higher AMF inoculum potential, than conventionally managed soil (Galvez et al. 2001; Oehl et al. 2003; Ozaki et al. 2004; Shrestha-Vaidya et al. 2008; Khanday et al. 2016), although, low input practices used in such management system do not always allow the level of biodiversity to increase, even after a long time (Franke-Snyder et al. 2001; Bedini et al. 2008). In a number of studies, organic management has been shown to stimulate AM fungi communities, with the effect attributed to reduced soil P under organic management (Ryan et al. 1994; Mader et al. 2000). This suggests that other practices such as the use of fertility building crops, a greater variety of cash crops, non-chemical weed control and non-use of fungicides may be important; all these factors are known to influence AMF populations (Kurle and Pfleger 1994). The relative difference in AMF spore numbers between organic and conventionally managed fields increased with time since conversion. Gryndler et al. (2009) studied that the mycelia of AM fungi are influenced by organic matter decomposition both via compounds released during the decomposition process and also by secondary metabolites produced by micro-organisms involved in organic matter (pure cellulose and alfalfa shoot and root material) decomposition.

21.7 Status Quo and Future Prospects of Mycorrhizal Application

Available summary of publications devoted to the agricultural and environmental benefits of mycorrhiza show their utility in the number cereal crops, fruits and vegetable production. Wheat, barley and paddy amongst cereals have been investigated with reference to effect of AM mycorrhiza on growth, productivity and nutrient uptake.

Attempts have been made to explore the possibility of employing AM technology in improving the production of vegetables, including potato, brinjal, tomato, lady's finger, lettuce, onion, pepper, cucumber, beans, tomato, muskmelon, watermelon, etc. Various fruit crops including citrus, papaya, orange, mulberry, apple and banana have been investigated for their response to inoculation of AM.

Arbuscular mycorrhizal symbiosis must be considered an essential factor for promoting plant health and productivity. Careful selection of compatible host/fungus/substrate combinations, would allow more appropriate management of mycorrhizae in poor soils would allow substantial reduction in the amount of minerals used without losses in productivity, while at the same time permitting a more sustainable production management.

Production of inoculum is expensive due to overhead and labor costs. During research work inoculum is prepared in an amount enough to treat a small research plot. Under field conditions application requires more inoculum than can be produced in pots. However, commercial inoculum production of AM fungi is under the process of improvement for the past decade, although the future prospect of the

business is still uncertain. With heightened interest in application of mycorrhizal fungi, due to their potential significance in not only sustainable crop production, but also in environmental conservation, it is likely that large scale production of inoculum would begin in the near future. Also, with the a basic understanding of the biology of AM fungi and refinement of application techniques for inoculum, the future of crop improvement and environmental conservation using mycorrhizal technology shows promise (Abbasi et al. 2015). Environmental stresses are becoming a major problem and productivity is declining at an unprecedented rate. Our dependence on chemical fertilisers and pesticides has encouraged the thriving of industries that are producing life-threatening chemicals and which are not only hazardous for human consumption but can also disturb the ecological balance. Biofertilizers can help solve the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realize the useful aspects of biofertilizers and implement its application to modern agricultural practices. The new technology developed using the powerful tool of molecular biotechnology can enhance the biological pathways of production of phytohormones. If identified and transferred to the useful PGPRs, these technologies can help provide relief from environmental stresses. However, the lack of awareness regarding improved protocols of biofertiliser applications to the field is one of the few reasons why many useful PGPRs are still beyond the knowledge of ecologists and agriculturists. Nevertheless, the recent progresses in technologies related to microbial science, plant–pathogen interactions and genomics will help to optimize the required protocols. The success of the science related to biofertilizers depends on inventions of innovative strategies related to the functions of PGPRs and their proper application to the field of agriculture. The major challenge in this area of research lies in the fact that along with the identification of various strains of PGPRs and its properties it is essential to dissect the actual mechanism of functioning of PGPRs for their efficacy toward exploitation in sustainable agriculture (Bhardwaj et al. 2014).

21.7.1 Improved Plant Nutrition

21.7.1.1 Phosphorus

Phosphorus is one of the most important nutrients for plant growth. Phosphorus is one of the least available and mobile plant nutrients in the soil (Takahashi and Anwar 2007). Many soils have high reserves of total phosphorus however only 0.1% of it is available to plants (Zou et al. 1992). At present 5, 49.3, 48.8 and 1.9% of Indian soils fall under adequate, low, medium and high categories of available phosphorus status respectively. The soils of Kashmir fall under low category of available phosphorus (Pattanayak et al. 2009). Inorganic fraction is an important form of phosphorus in soils. It is generally categorized into insoluble and readily soluble categories. The insoluble fraction is neither available to growing plants nor

to microorganisms and constitutes 94–99% of the total soil phosphorus. This fraction is mostly attached to Fe and Al in acid soils and to calcium in slightly acidic to alkaline soils. Inorganic phosphates when applied to soil get transformed to various reaction products mainly remaining in sparingly soluble orthophosphates of Al, Fe and Ca. Therefore costly phosphatic fertilizers have to be applied to the agricultural fields to maximize production. However, the soluble phosphorus in these fertilizers is easily and rapidly precipitated to insoluble forms with cation such as Ca^{2+}, Fe^{3+}, Al^{3+} or Zn^{2+} or adsorbed to calcium carbonate, aluminum oxide, iron oxide and aluminum silicate so the apparent recovery of applied phosphorus in soils is very less i.e. 15–20%. This transformation decreases the efficiency with which soluble phosphorus can be taken up by the plants and decreases the effectiveness of the fertilizer resulting in the application of increasing amounts of phosphatic fertilizers to the agricultural fields. This unmanaged use of phosphatic fertilizers has increased agricultural costs and instigated a variety of environmental and health hazards as these contain potentially toxic heavy metals (Pb, Cd, As etc). Their excessive use has rendered the fertile soils sick by disturbing the soil microbial biodiversity. Therefore the use of AM in organic agriculture for enhancing host plant phosphorus nutrition is economically and environmentally promising strategy. There are four general assumptions associated with the improved host plant phosphorus nutrition.

- The external mycelium of mycorrhizal fungus can take up P in the form of trehelose phosphate more effectively than roots at low concentrations.
- The external mycelium can proliferate for beyond the rhizosphere and increases the soil volume which is exploited for phosphorus uptake. The hyphal transport of phosphorus has been estimated to be 20–90% and likely to fulfill the entire requirement of fertilizer phosphorus.
- Rapid absorption of soluble form of phosphorus by the external hyphae leads to a shift in equilibrium towards the release of bound phosphorus from soil reserves (Smith and Read 1997).
- Mycorrhizal roots of onion increased the acid phosphatase activity by 20–30 times in comparison to non-mycorrhizal roots that catalyze the hydrolysis of complex insoluble phosphorus compounds in the soil and increase the soluble form of phosphorus.

These mechanisms aid in the uptake of phosphorus by the host plants and help in reducing the dependence on inorganic phosphatic fertilizers. Thus mycorrhiza plays a pivotal role in the solubilization, mobilization and uptake of phosphorus and can be exploited in organic farming up to the fullest possible extent.

21.7.1.2 Nitrogen

Nitrogen has the distinction among all the essential nutrients of being called as "Kingpin" nutrient. Its use is indispensible in low as well as conventional production systems. The available nitrogen status in agricultural soils is subjected to

various losses through the processes like leaching and volatilization. Under such conditions mycorrhizal fungi play a significant role in improving nitrogen nutrition of plants through acquisition and assimilation mechanisms.

- The external fungal mycelium plays an important role in direct nitrogen acquisition and transport to the root cells thereby contributing to plant nutrition. Studies by Fray and Schuepp (1993) have revealed that the extraradical mycelium in mycorrhizal fungi can derive ^{15}N from the soil. Subramanian and Charest (1999) in a box compartmental experiment have shown that the amount of nitrate (NO_3^-) ions being transported by the external hyphae was about 30–35% under water deficient conditions.
- Mycorrhizal colonization of roots has increased the activities of nitrogen assimilatory enzymes such as nitrogen reductase (NR), glutamine synthetase (GS), and glutamate synthase (GOGAT) in drought stressed maize roots (Subramanian and Charest 1998, 1999).
- Under soil conditions where less mobile ammonium ions are dominant the role of mycorrhizal symbiotic association becomes more important.
- Mycorrhizal fungi enters in tripartiate association (Soybean-*Rhizobium-Glomus*) thereby aids in transfer of nitrogen fixed by *Rhizobium* to the non-leguminous neighboring plants.

These evidences suggest that mycorrhizal fungi can successfully be exploited for improvement in nitrogen nutrition of crop plants under organic farming.

21.7.1.3 Micronutrient Nutrition

The external hyphae explores the soil beyond the root hair zone and thereby increasing plant growth by enhancing uptake of diffusion limited nutrients. Mycorrhizal hyphae develop intensively inside the roots and with in the soil forming extensive extraradical which help the plant in exploiting mineral nutrients and water from the soil. In plants particularly those with week/restricted root system, hyphal connections act as a bridge between roots and nutrient sites in soil and facilitate efficient uptake of immobile nutrients by host plant. Depending up on the host plant, colonization by mycorrhizal fungi can increase nutrition of micronutrients especially Zn in addition to Cu, Mn and Fe (Rupam et al. 2008; Khanday et al. 2016). Among the essential nutrients required by crops, zinc is considered the most critical micronutrient causing yield reduction to the tune of 10–50% depending on the severity and stage of occurrence. The magnitude of Zn deficiency is high in almost every type of soil and the major portion of added Zn gets fixed. Further, imbalanced use of fertilizers and non-addition of organic manures are believed to be aggravating the situation. In some cases, zinc deficiency in soil reduces grain yield up to 80% along with reduction in grain Zn content and other nutritional qualities. High dependence on cereal based diets with low levels of Zn brings out malnutrition of human beings and globally, over two billion people are affected by Zn deficiency. Improving food grain production with nutritionally rich

grain quality is the need of the hour to sustain grain production and to ensure nutritional security. Despite the fact that importance of Zn nutrition is well known, it is very difficult to ameliorate Zn deficiency in crops due to the extremely low use efficiency of zinc (<1%) by crops and the remaining 99% get fixed in the soil. Indeed, majority of arable lands have high total Zn but the bioavailability is too low, suggesting that there is a need to adopt strategies to transform the unavailable form to available form of Zn. One of the biological means to mitigate Zn deficiency is by exploiting naturally occurring mycorrhizal symbiosis. Arbuscular mycorrhizal (AM) fungi immobile micronutrients such as Zn and Cu.

- Mycorrhizal fungi lower the pH around the rhizosphere that helps in release of Zn from the fixed pool
- The external mycelium of mycorrhizal fungi is very explorative and transport Zn far from the root zone to the tune of 40% contributing for the host plant nutrition.
- Rhizosphere of mycorrhizal roots are biochemically active in term of soil enzymes and release a specific glomalin protein that serves as adsorptive site for Zn which in turn is made available to the host plant.
- Interestingly, mycorrhizal fungi are able to extract Zn from tightly bound residual form of Zn and contribute for the organic bound and water soluble forms of Zn. As the result of these mechanisms and processes, mycorrhizal plants are more efficient in utilizing the Zn from the soil and help the plants to produce higher grain yield by 10–15%. Thus mycorrhizal inoculation is one of the potential strategies to improve Zn use efficiency by crops besides enhancing the yield and quality of grains.

21.7.1.4 Plant Protection

Mycorrhizal fungi have been well documented as biocontrol agents and the general conclusion is that they can reduce or even suppress damage caused by soil borne pathogen (Khanday et al. 2016). AMF colonized plants have shown a significant degree of bioprotection against various pathogens like *Fusarium, Pythopthora, Aphanomyces, Verticillium* (Elsen et al. 2001; Azcon and Barea 1996) and nematodes causing respectively root rot, lesions, wilt and galls (Guillemin et al. 1994). Several genes and corresponding protein products involved in plant defense responses have been extensively studied in AMF symbiosis and have been shown to be spatially and temporally expressed (Harrier and Watson 2004). These include callose deposition, phytoalexins, β-1-3 glucanases, chitinases and PR pathogenesis related proteins (Guillon et al. 2002). Cordire et al. (1996) showed that pre-inoculation of tomato with an AM fungus subsequently challenged by *Pythopthora parasitica* resulted in less root damage. In that study the authors used immunogold labelling technique to show that the number of hyphae of the pathogen was greatly reduced in mycorrhizal roots and mycorrhizal root tissues infected by the pathogen. The AMF was able to confer bioprotection against *Phytophthora parasitica via* localized and induced systemic resistance in mycorrhizal and non mycorrhizal roots respectively.

21.7.1.5 Alleviation of Environmental Stresses

Mycorrhization with arbuscular mycorrhizae enable plants to tolerate a wide range of environmental stresses such as drought, toxic metals, saline soil, root pathogens, high soil temperature and adverse pH (Caldwell and Virginia 1989). A well developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by improving water relations, better nutrient acquisition, pathogenic resistance, amelioration of soil structure, phytohormone production and contribution to soil aggregation thus improving the success of all kinds of bioremedation such as decreased caesium uptake by AMF treated plants and can be used effectively in the establishment of plant cover on radionucleide contaminated soils, thereby reducing environmental risks. Mycorrhization can also be used for attenuation of deleterious soil conditions. They also have the potential to monitor site toxicity or the efficiency of restoration techniques (Weissenhorn et al. 1993). Therefore mycorrhizal fungi enable plants to cope with abiotic stresses by alleviating mineral deficiencies, overcoming the detrimental effects of salinity, improving drought tolerance, enhancing tolerance to pollution and improving the adaptation of sterile micropropagated plantlets to cope up with sudden stress situations arising as a result of change in environmental conditions encountered as a result of their shift from in vitro to in vivo conditions (Barea et al. 1993). Mycorrhizae protects the plants from adverse impact of heavy metals by following mechanisms:

Biosorption by Mycorrhizal Fungi

- Adsorption: Fungal wall (chitin) binds the metals.
- Complexation: Organic acids produced by mycorrhizae forms complex with heavy metals.
- Precipitation: Formation of intra cellular heavy metal phosphates.

Detoxification Mechanism

- Avoidance: Some times mycorrhizal mycellium avoids the absorption of metal ions.
- Solubilization: Dilution of metals.

Arbuscular Mycorrhizal Inoculation

Optimum spore count : 60–100 spores/100 g soil

Rate of Inoculation

Vegetables	:	100 g/m^2 nursery
Fruit trees and apple	:	100–200 g/tree
Other crops	:	10% of seed rate

21.8 Methods of Inoculum Production

The thresh hold point related to the use of AM fungi as plant growth promoters is the development of suitable techniques for the production of large quantities of pure pathogen free inoculum with high infectivity potential. Some of the commonly used methods for mass production of AM spores are listed below:

21.8.1 Soil Based Inocula

21.8.1.1 Pot Culture

It is the most widely used standard and conventional method of maintaining AM fungal cultures around the world. In this method AMF spores are inoculated to the roots of a trap plant raised on sterilized soil. Though the usual substrate used in pot culture is sterilized sand-soil mixture, sometimes inorganic inert material like peat, perlite and vermiculite can be also used as substrate (Abdul Khaliq et al. 2001). The trap plants commonly used for pot culture are *Sorghum halepense, Paspalum notatum, Panicum maxicum, Cenchrus ciliaris, Zea mays, Trifolium subterraneum* and *Allium cepa* (Chellapan et al. 2001). The inoculum so produced, consists of a mixture of soil, spores, hyphal segments and infected root pieces and generally takes around 3–4 months.

21.8.1.2 Inoculum Rich Soil Pellets

A technique of AMF inoculum production, in which soil pellets are enriched with the AMF inoculum was introduced by Hall and Kelson (1981). The pellets had an average dry weight of 1.55 g and measured 12 × 12 × 6 mm. These dry pellets can be glued with seeds by gum Arabic and can easily be broadcasted like other fertilizers and spread during seed sowing or transplantation.

21.8.2 Soil Free Inocula

21.8.2.1 Aeroponic Culture

Apart from soil based pot cultures being the most widely used method for AMF inoculum production. Now a days, for physiological, genetic studies for in vitro mycorrhization, the focus is shifting towards alternative soil less cultures for mass production of clean and pure AMF propagules (Mohammad et al. 2000). In aeroponic cultures, pure and viable spores of a selected fungus are used to inoculate the cultured plants, which are later transferred in to a controlled aeroponic chamber

(Singh and Tilak 2001) where the nutrient solution is provided in the form of a mist. Lack of physical substrate ensures extensive root growth, colonization and sporulation of the fungus and makes it an ideal system for obtaining sufficient amounts of clean AMF propagules (Abdul Khaliq et al. 2001).

21.8.2.2 Root Organ Culture

The main obstacle in the study of AMF and AMF symbiosis are the obligate biotrophic and hypogeal nature of the endophyte. Several attempts have been made in the past to overcome these hurdles through the use of *in vitro* root organ culture, because of its potential for research and inoculum production. *Agrobacterium rhizogenes* is a Gram negative soil inhabiting bacteria, which produces a condition called "hairy roots" as a result of the modified hormonal balance of the tissues that makes them vigorous and allows it to grow rapidly on artificial media (Abdul Khaliq et al. 2001). Once the hairy roots are ready, spores are collected either from field or from pot cultures by wet sieving and decanting method (Gerdemenn and Nicolson 1963). Generally two types of fungal inoculum are used for initiating monoxenic cultures; extraradical spores or mycorrhizal root fragments and isolated vesicles of the fungus. In addition to the spores and root fragments, sporocarps of *Glomus mosseae* have also been used by Budi et al. (1999) to establish *in vitro* cultures. After isolating the fungus from the soil, spores are surface sterilized using a suitable surfactant solution. Generally between 20 and S solution containing a strong oxidizing agent chloramine T are used for sterilization of AMF spores (Fortin et al. 2002). Then the spores are subsequently rinsed thoroughly in streptomycin-gentamycin antibiotic solution (Becard and Piche 1992). All steps starting from spore isolation to rinsing should be done on ice, to maintain spore dormancy. The rinsed spores should be stored at 4 °C in distilled water or water agar or on 0.1% $MgSO_4 \cdot 7H_2O$ solidified with gelatin gum, if not used immediately (Fortin et al. 2002).

The final step in raising a successful *in vitro* culture is the selection of the appropriate culture medium for dual cultivation of the partners, the host root and the AMF. The nutrient media should be carefully selected to allow the growth of the host as well as the fungus during the dual culture establishment. Since the root needs rich nutrient medium for its growth and the AMF require normally a relatively poor nutrient medium (Abdul Khaliq et al. 2001). Generally, Murashige and Skoog's (1962) and White's medium are used for establishing the dual culture of the host root and the AMF symbionts.

21.8.2.3 Nutrient Film Technique

The NFT is another technique of soil less inoculum production, pioneered by Cooper (1975). In NFT, the plant roots are provided with a shallow layer of rapidly flowing nutrient solution. As a result of it, root mats are formed and the upper layer

above the liquid retains a film of moisture around them. The pre inoculated seedlings are planted in to the NFT unit. The inoculum produced by this method is ideal for the production of easily harvestable solid mats of roots with more concentrated and less bulky form of inoculum than that produced by plants grown in soil based or other solid media (Abdul Khaliq et al. 2001).

21.8.2.4 Polymer Based Inoculum

Encapsulation or entrapment of AMF in polymer materials is frequently used as a powerful means of immobilization. It includes the encasement of AMF spores, vesicles or mycorrhizal roots within a porous structure formed '*in situ*' around the biological material. In polymer based inoculum preparation, the AMF are generally mixed with a compound which is then gelled to form a porous matrix under conditions sufficiently mild, so as not to affect the viability of biological material. Around 1350 combinations of natural, semi synthetic and synthetic polymers exist for entrapment of AMF (Vassilev et al. 2005). But the majority of techniques involving '*in situ*' entrapment make use of natural polysaccharide gels including kappa-carrageeman, agar and alginates. Calcium alginate is the most widely used carrier of choice for encapsulation of AMF. In some cases spores of AMF can be introduced directly in synthetic seeds, which can germinate under suitable conditions and can become complete plantlets.

21.9 Techniques to Observe AM Fungi

Most observations of mycorrhizae are based on the use of Trypan blue (0.05%) to stain fungi in host roots (Phillips and Hayman 1970). In this technique the mycorrhizal roots are treated in hot 10% KOH that first removes the host cytoplasm and then the nuclei. After the roots are neutralized in a weak acid wash, they are stained in Trypan blue. The stain penetrates deeply and usually stains the hyphae but does not deeply stain the plant tissue. This technique generally is satisfactory for agronomic crops and many other species.

Kormanik et al. (1980) described an Acid Fuschin technique in which clearing and staining of many plant root samples for observation can be accomplished. This technique produces more satisfactory results in plants with heavy pigmented roots. Brundrett et al. (1984) developed another technique in which chlorazol black E allowed the detection of the developmental stages of AM fungi in the host roots with more clarity than other techniques. There are problems with all these techniques. All the techniques are destructive to the sample and involve time-consuming procedures. Different taxa are stained with different intensities in the same roots. Many species of *Gigaspora* and *Scutellospora* stain intensely with Trypan blue, regardless of the host species (Morton 1988). *Acaulospora trappei* exhibits intermediate staining in Trypan blue (Abbott 1982). *Glomus dimorphism,*

G. fecundisporum, G. leptoticum, G. maculosum, G. occultum, G. tortuosum, Acaulospora myriocarpa, and *Entrophospora schenckii* are not stained or are weakly stained in Trypan blue (Morton 1985). The variation in staining may leave regions unstained and cause inaccurate estimations of fungal colonization of a root. Ames et al. (1982) developed a nondestructive approach to estimate fungal metabolic activities in structures within and outside the host roots. This technique depends on using fluorescein diacetate (FDA) as a non-polar molecule that is taken up by the fungus. If the proper enzymes are present, FDA is hydrolyzed, and fluoresce accumulates in the cell. When excited with ultraviolet (UV) light (450–490 nm), becomes fluorescent and emits at 520–560 nm. The problem with this technique is that much of the hyphae, vesicles, and intra radical spores are not visible. A further problem is that suberized or lignified root tissue may occlude the fungal structures and auto fluorescence.

It can be concluded here that AM fungi can be used quite successfully as a nutrient input under low input agriculture production system.

21.10 Benefits

21.10.1 Soil Structure

Arbuscular mycorrhizae are important factors of soil quality through their effects on host plant physiology, soil ecological interactions and their contributions to maintaining soil structure (Rillig 2004). Mycorrhizal filaments produce humic compounds and organic glues (extracellular polysaccharides) that bind soil into aggregates and improves soil porosity. Soil porosity and soil structure positively influence the growth of plants by promoting aeration, water movements into soil, root growth and distribution. In sandy or compacted soils, the ability of mycorrhizal fungi to promote soil structure may be more important than the seeking out of nutrients.

21.10.2 Plant Growth Hormones

Certain AMF spores or seeds of the fungus have been selected for their establishment and growth-enhancing abilities. Mycorrhizal inoculants can be sprinkled onto roots during transplanting, worked into sed beds, blended into potting soil, watered in via existing irrigation systems, applied as a root dip gel or probed into the root zone of existing plants. AM fungi also increase the production of plant growth hormones such as cytokinins and gibberellins.

21.10.3 Plant Roots

AM fungi increase overall absorption capacity of roots due to morphological and physiological changes in the plant. There is increased absorption surface area, greater longevity of absorbing roots, better utilization of low-availability nutrients and better retention/storage of nutrients, thus reducing reaction with soil colloids or leaching losses. Nodulation and atmospheric nitrogen fixation capacity in legumes were also increased by AM fungi.

21.10.4 Crop Yield

Mycorrhizal fungi improve crop yields (Siddiqui and Mahmood 2001), especially in infertile soil (Hayman 1982). Many crops are grown in acid soil, where their establishment is frequently limited by low availability of phosphorus. In this case, appropriate mycorrhizal fungi can greatly improve crop yields by increasing the phosphorus uptake by plants (Howeler et al. 1987). When the availability in soil is low, non-mycorrhizal root systems may be unable to absorb P effectively and the plants become P deficient and grow poorly. AM colonization and P uptake lead to relief of this nutrient stress and, in consequence, plant growth is increased. This is the well-known mycorrhizal growth response (the big and little plant effect) which has been demonstrated for an enormous number of species mainly in pot experiments.

21.10.5 Nutrient Uptake

It is now established that the fungal partner can make a considerable contribution to nutrient uptake. AM fungi can mediate inter-plant transfer of phosphorus (Francis et al. 1986; Newman and Ritz 1986), carbon (Newman 1988; Read et al. 1985) and nitrogen (Read et al. 1985; Kessel et al. 1985; Haystead et al. 1988; Barea et al. 1988; McNeil and Wood 1990). The largest effect of AM formation is on P nutrition. In addition to phosphorus uptake, AM fungi can also enhance the uptake of relatively immobile micro nutrients, particularly zinc and copper (Killham and Firestone 1983; Lambert et al. 1979; Gnekow and Marschner 1989; Gildon and Tinker 1983;Pacovsky 1986).

21.10.6 Disease and Pathogen

AM fungi are recognized as high potential agents in plant protection and pest management (Quarles 1999; Sharma and Dohroo 1996; St-Arnaud et al. 1995).

Mycorrhizal roots have a mantle that acts as a physical barrier against the invasion of root diseases AMF secretes antibiotics that competes or antagonizes pathogens, thus aiding in disease suppression. AM fungi can decrease the severity of diseases caused by root pathogenic fungi, bacteria and nematodes (Jalali and Chand 1988; Siddiqui and Mahmood 1995a, b; Bhat and Mahmood 2000; Shafi et al. 2002). In several cases direct biocontrol potential has been demonstrated, especially for plant diseases caused by *Phytophthora*, *Rhizoctonia* and *Fusarium* pathogens (Siddiqui and Mahmood 1996; Abdelaziz et al. 1996; St-Arnaud et al. 1997; Siddiqui et al. 1998; Dalpe and Monreal 2004).

21.10.7 Weed Control

Mycorrhizal fungi can contribute to weed control also. They suppress the competitive ability of weeds relative to sunflower (Van der Heijden et al. 2008). AM fungi have the potential to be a much more environmentally sound method of *Poa annua* (weed of temperate zone golf) control in sports turf than the currently used chemicals (Gange et al. 1999). Btehlenfalvay et al. (1996) studied that mycorrhizal fungi enhance weed control and crop growth in a soybean-cocklebur association treated with herbicide bentazon.

21.10.8 Land Rehabilitation

The effective role of AM fungi in land rehabilitation has been well documented (Allen and Allen 1988; Sylvia and Will 1988; White et al. 1989). The AM fungi, by maintaining the uptake of slowly diffusing nutrients under water stress conditions, can help plants resist drought stress (Azcon et al. 1988). AM fungi can help plants become established in saline soils (Hirrel and Gerdemann 1980; Pond et al. 1984) and in nutrient deficient soil or degraded (eroded) habitats, in coal wastes, eroded desert and disturbed soils (Hall and Armstrong 1979; Khan 1981). Mycorrhizal fungi appear to have beneficial effects on soil aggregation and may be an important means of controlling soil erosion. Extramatrical mycelia of AM fungi have been reported to bind soil grains in sandy soils and dunes and many sand dune plants are known to be mycorrhizal.

21.11 Conclusion

Biofertilizer help in increasing crop productivity by way of increased biological nitrogen fixation, increased availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth through hormonal action or

antibiosis, or by decomposition of organic residues. Furthermore, biofertilizer as to replace part of the use of chemical fertilizers reduces amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers. With using the biological and organic fertilizers, a low input system can be carried out, and it can be helped achieving sustainability of farms. Worldwide, considerable progress has been achieved in the area of mycorrhizal technology. Mycorrhizal fungi are one of the more important groups of soil organisms and play a critical role in nutrient cycling, mediating plant stress and protection against pathogens. They are also cornerstones in the ability of plants to survive transplant shock. Plants have co-evolved mutualistic relationships with symbiotic mycorrhizal fungi such that their survival and fitness depends upon the healthy functioning of these fungi and *vice-versa*. The evidence suggests that the organic farming system leads to increase the inoculum levels of AMF with greater crop colonization that resulted in enhanced nutrient uptake and therefore mycorrhiza may be used as a substitute to reduced fertilizers.

It has been demonstrated and proved that mycorrhizae have great potential for field application to improve productivity of cereal, fruit and vegetable crops and suppress nematode and fungal infestations. The public demand to reduce environmental problems associated with excessive pesticide usage has prompted research on reduction or elimination of pesticides and increasing consumer demands for organic or sustainably-produced food requires the incorporation of microorganisms, such as arbuscular mycorrhizal (AM) fungi. There is also an urgent need to strengthen further the regional collaboration so that benefits of technology advancements could reach those presently left behind.

References

Abbasi, Hisamuddin, Akhtar A, Sharf R (2015) Vesicular Arbuscular mycorrhizal (VAM) fungi: a tool for sustainable agriculture. Am J Plant Nutr Fertil Technol 5:40–49

Abbott LK (1982) Comparative anatomy of vesicular-arbuscular mycorrhizae formed on subterranean clover. Aust J Bot 30:485–499

Abdelaziz RA, Radwansamir MA, Abdel-Kader M, Barakat MA (1996) Biocontrol of faba bean root-rot using VA mycorrhizae and its effect on biological nitrogen fixation. Egypt J Microbiol 31:273–286

Abdul Khaliq, Gupta ML, Alam A (2001) Biotechnological approaches for mass production of arbuscular mycorrhizal fungi: current scnerio and future strategies. In: Mukerji KG, Manoharachary C, Chamola BP (eds) Technique in mycorrhizal studies. Kluwer Academic Publishers, The Netherlands, pp 299–312

Allen EB, Allen MF (1988) Facilitation of succession by the non-mycotrophic colonizer *Salsola kali* (Chenopodiaceae) on a harsh site: effects of mycorrhizal fungi. Am J Bot 75:257–267

Allen MF, Moor TSJ, Christensen M (1982) Phytohormone change in *Bouteloua gracilis* infected by vesicular arbuscular mycorrhiza II: altered levels of gibberellin-like substances and abscisic acid in the host plant. Can J Bot 60:468–471

Ames RN, Ingham ER, Reid CPP (1982) Ultraviolet-induced autofluorescence of arbuscular mycorrhizal root infections: an alternative to clearing and staining methods for assessing infection. Can J Microbiol 28:351–355

Asai T (1944) Uber die Mykorrhizenbildung der leguminosen Pflanzen. Jpn J Bot 13:463–485
Auge RM, Duan X, Ebel RC, Stodala AJW (1994) Non-hydraulic signalling of soil drying in mycorrhizal maize. J Planta 193:74–82
Azcon AC, Barea JM (1996) Arbuscular mycorrhizae and biological control of soil borne plant pathogens: an over view of the mechanism involved. Mycorrhizae 6:457–464
Azcon R, El-Atrach F, Barea JM (1988) Influence of mycorrhiza vs. soluble phosphate on growth, nodulation and N_2 fixation (^{15}N) in Alfalfa under different levels of water potential. J Biol Fertil Soils 7:28–31
Azcon-Aguilar C, Azcon R, Barea JM (1979) Endomycorrhizal fungi and *Rhizobium* as biological fertilizers for *Medicago sativa* in normal cultivation. Nature 279:325–327
Bagyaraj DJ (1989) Mycorrhizae. In: Tropical rain forest ecosystems. Elsevier Science Publishers, Amsterdam, pp 537–546
Bagyaraj DJ, Varma V (1995) Interaction between arbuscular fungi and plants: their importance in sustainable agriculture and in arid and semi arid tropics. In: Advances in microbial ecology. Academic Press, London, pp 119–142
Barea JM, Jeffries P (1995) Arbuscular mycorrhizas in sustainable soil plant systems. In: Hock B, Varma A (eds) Mycorrhiza structure, function, molecular biology and biotechnology. Springer, Heidelberg, pp 521–559
Barea JM, Azcon-Aguilar C, Azcon R (1988) The role of mycorrhiza in improving the establishment and function of the *Rhizobium* under field conditions. In: Beck DP, Materon LA (eds) Nitrogen fixation by legumes in mediterranean agriculture. ICARDA and Martinus Nijhoff Dordrecht, Berlin, pp 153–162
Barea JM, Azcon R, Azcon AC (1993) Mycorrhiza and crops. In: Tommerup I (ed) Advances in plant pathology, mycorrhiza: a synthesis, vol 9. Academic Press, London, pp 167–189
Becard G, Piche Y (1992) Establishment of vesicular arbuscular mycorrhiza in root organ culture: review and proposed methodology. In: Norris et al (eds) Methods in microbiology, vol 24. Academic Press, London, pp 89–108
Bedini S, Cristani C, Avio L, Sbrana C, Turrini A, Giovannetti M (2008) Influence of organic farming on arbuscular mycorrhizal fungal populations in a Mediterranean agro-ecosystem. In: Proceedings of 16th IFOAM organic world congress, June 16–20, Modena, Italy
Bending GD, Turner MK, Rayns FR, Marx MC, Wood M (2004) Microbial and biochemical indicators of soil quality and their potential for differentiating areas under contrasting agricultural management regimes. J Soil Biol Biochem 36:1785–1792
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. Microbial Cell Fact 13:66. https://doi.org/10.1186/1475-2859-13-66
Bhat MS, Mahmood I (2000) Role of *Glomus mosseae* and *Paecilomyces lilacinus* in the management of root knot nematode on tomato. Arch Phytopathol 33:131–140
Brundrett MC, Piche Y, Peterson RL (1984) A new method for observing the morphology of vesicular arbuscular mycorrhizae. Can J Bot 62:2128–2134
Btehlenfalvay GJ, Schreiner RP, Mihara KL, McDaniel H (1996) Mycorrhizae, biocides and biocontrol. 2. Mycorrhizal fungi enhance weed control and crop growth in a soybean-cocklebur association treated with the herbicide bentazon. J Appl Soil Ecol 3:205–214
Budi SW, Blal B, Gianinazzi S (1999) Surface sterilization of *Glomus mosseae* sporocarps for studying endomycorrhization in vitro. Mycorrhiza 9:65–68
Caldwell MM, Virginia RA (1989) Root systems. In: Pearcy RW, Ehleringer JA, Mooney HA, Rundel PW (eds) Plant physiological ecology-field methods and instrumentation. Chapman and Hall, London, pp 367–398
Celik I, Ortas I, Kilic S (2004) Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a chromoxerert soil. Soil Tillage Res 78:59–67
Chaudhari D (2015) A short review on polonium as a carcinogen in tobacco. Int J Adv Res 3:1092–1093
Chellapan P, Chrasty SAA, Mahadevan A (2001) Multiplication of mycorrhizaon roots. In: Mukerji KG, Manoharachary C, Chamola BP (eds) Techniques in mycorrhizal studies. Kluwer Academic Publishers, The Netherlands, pp 285–297

Chuck S (2008) Screening evaluation of heavy metals in inorganic fertilizers. Minnesota Department of Health, St Paul, MN, p 26
Cooper AJ (1975) Crop production in there circulating nutrient solutions. Sci Hortic 3:251–258
Cordire C, Gianinnzi P, Gianinnzi S (1996) Colonization patterns of root tissues by *Phytophthora nicotiana* var. parasitica related to reduced disease in mycorrhizal tomato. Plant Soil 185:223–232
Dalpe Y, Monreal M (2004) Arbuscular mycorrhiza inoculum to support sustainable cropping systems. Crop Manage. https://doi.org/10.1094/CM-2004-0301-09-RV
Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of mycorrhiza colonizing arable crops. FEMS Microbiol Ecol 36:203–209
Elsen A, Declerck S, Waele D (2001) Efect of *Glomus intraradicies* on the reproduction of burrowing nematodes (*Rhadopholus similis*) in dixenic culture. Mycorrhiza 11:49–51
Ezawa T, Yamamoto K, Yoshida S (2000) Species composition and spore density of indigenous vesicular-mycorrhiza under different conditions of P fertility as revealed by soybean trap culture. J Soil Sci Plant Nutr 46:291–297
Fortin JA, Becard G, Declerck S, Dalpe Y, Arnaud SM, Coughlan AP, Piche Y (2002) Arbuscular mycorrhiza on root organ cultures. Can J Bot 80:1–20
Francis R, Finlay RD, Read DJ (1986) Vesicular-arbuscular mycorrhiza in natural vegetation systems. VI. Transfer of nutrients in inter and intra-specific combinations of host plants. J New Phytol 120:103–111
Franke-Snyder M, Douds DD, Galvez L, Phillips JG, Wagoner P, Drinkwater L, Morton JB (2001) Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA. J Appl Soil Ecol 16:35–48
Fray B, Schuepp H (1993) Acquisition of N by external hyphae of AM fungi associated with maize. New Phytol 124:221–230
Galvez L, Douds DD, Drinkwater JLE, Wagoner P (2001) Effect of tillage and farming system upon VAM fungus populations and mycorrhizas and nutrient uptake of maize. J Plant Soil 228:299–308
Gange AC, West HM (1994) Interactions between mycorrhiza and foliar-feeding insects in *Plantago lanceolata* L. New Phytol 128:79–87
Gange AC, Lindsay DE, Ellis LS (1999) Can mycorrhizabe used to control undesirable grass *Poa annua* on golf courses. J Appl Ecol 36:909–919
Gaur R, Shani N, Kawaljeet K, Johri BN, Rossi P, Aragno M (2004) Diacetylchloroglucinol-producing pseudomonads do not influence AM fungi in wheat rhizosphere. Curr Sci 86:453–457
Gerdemenn JV, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Brit Mycol Soc 46:235–244
Gildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infections and heavy metals in plants. New Phytol 95:263–268
Giovannetti M, Gianinazzi-Pearson V (1994) Biodiversity in arbuscular mycorrhizal fungi. J Mycol Res 98:705–715
Gnekow MA, Marschner H (1989) Role of VA-mycorrhiza in growth and mineral nutrition of apple (*Malus pumila* var. Domestica) rootstock cuttings. J Plant Soil 119:285–293
Gosling P, Shepherd M (2005) Long term changes in soil fertility in organic farming systems in England, with particular reference to phosphorus and potassium. Agric Ecosyst Environ 105:425–432
Gosling P, Hodge A, Goodlass G, Bending GD (2006) Mycorrhizaand organic farming. Agric Ecosyst Environ 113:17–35
Gryndler M, Hrselova H, Cajthaml T, Havrankova M, Rezacova V, Gryndlerova H, Larsen J (2009) Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. Mycorrhiza 19:255–266
Guillemin JP, Gianinazzi P, Marchal J (1994) Contribution of mycorrhizas to biological protection of micropropagated pine apple (*Ananas comosus* (L) Merr) against *Phthopthora cinnamomi* Rads. Agric Sci Finl 3:241–251

Guillon C, Arnod STM, Hamel C, Jabaji HSH (2002) Differential and systemic alteration of defence related gene transcript levels in mycorrhizal bean plants with *Rhizoctonia solani*. Can J Bot 80:305–315

Hall IR, Armstrong P (1979) The effect of vesicular-arbuscular mycorrhizas on growth of clover, lotus and ryegrass in some eroded soils. J Agric Res 22:479–484

Hall IR, Kelson A (1981) An improved technique for the production of endomycorrhizal infested soil pellets. N Z J Agric Res 24:221–222

Hamel C, Dalpe Y, Lapierre C, Simard RR, Smith DL (1996) Endomycorrhiza in a newly cultivated acidic meadow: Effects of three years of barley cropping, tillage, lime and phosphorus on root colonization and soil infectivity. Biol Fertil Soils 21:160–165

Harrier LA, Watson CA (2004) The potential role of mycorrhizain the bio protection of plants against soil borne pathogens in organic and/or sustainable farming systems. Pest Manag Sci 60:149–157

Hayman DS (1982) Practical aspects of vesicular-arbuscular mycorrhiza. In: Subbra-Rao NS (ed) Advances in agricultural microbiology. Oxford and IBM Publishing Company, New Delhi, pp 325–373

Hayman DS (1986) Mycorrhizae of nitrogen fixing legumes. World J Microbiol Biotechnol 2:121–145

Haystead A, Malajczuk N, Grove TS (1988) Underground transfer of nitrogen between pasture plants infected with vesicular-arbuscular mycorrhizal fungi. New Phytol 108:417–423

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web. Nature 394:431–431

Hirrel MC, Gerdemann JW (1980) Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. Am J Soil Sci 44:654–658

Hirrel MC, Mehravaran H, Gerdemann JW (1978) Vesicular mycorrhiza in the Chenopodiaceae and Cruciferae: do they occur? Can J Bot 56:2813–2817

Hodge A (2000) Microbial ecology of the arbuscular mycorrhiza. FEMS J Microbiol Ecol 32:91–96

Howeler RH, Sieverding E, Saif F (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. Plant Soil 100:249–283

IFOAM (1998) Basic standards for organic production and processing. IFOAM Publications, Germany

Jalali BL, Chand H (1988) Role of VAM in biological control of plant diseases. In: Mohadevan A, Raman N, Natarajan K (eds) Mycorrhizae for Green Asia. Madras Express Service, India, pp 209–215

Jeffries P, Barrea JM (1994) Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systems. In: Gianinazzi S, Schuepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhauser Publisher, Basel, pp 101–115

Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS J Microbiol Ecol 48:1–13

Joner EJ (1996) Mycorrhiza in organic farming E8. In: Proceedings of the 11th IFOAM scientific conference, 11–15 August, Copenhagen, Denmark

Kabir Z, O'Halloran IP, Fyles JW, Hamel C (1998) Dynamics of the Mycorrhizal symbiosis of corn (*Zea mays* L.): effects of host physiology, tillage practice and fertilization on spatial distribution of extra-radical mycorrhizal hyphae in the field. J Agric Ecosyst Environ 68:151–163

Kessel CV, Singleton PW, Hoben HJ (1985) Enhanced N-transfer from soybean to maize by vesicular-arbuscular mycorrhizal (VAM) fungi. J Plant Physiol 79:562–563

Khan AG (1981) Growth response of endomycorrhizal onions in non-sterilized coal waste. New Phytol 87:363–370

Khanday M, Bhat RA, Haq S, Dervash MA, Bhatti AA, Nissa M, Mir MR (2016) Arbuscular mycorrhizal fungi boon for plant nutrition and soil health. In: Hakeem KR et al (eds) Soil

science: agricultural and environmental prospectives. Springer International Publishing, Switzerland, pp 317–332
Khosro M, Yousef S (2012) Bacterial biofertilizers for sustainable crop production: a review. J Agric Biolog Sci 7: 307–316
Killham K, Firestone MK (1983) Vesicular-arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. J Plant Soil 72:39–48
Kormanik P, Bryan WC, Schultz RL (1980) Procedure and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can J Microbiol 26:536–538
Kurle JE, Pfleger FL (1994) The effects of cultural practices and pesticides on VAM fungi. In: Pfleger FL, Linderman RG (eds) Mycorrhizae and plant health. APS Press, St. Paul, MN, pp 101–131
Lambert DH, Baker DE, Cole H (1979) The role of mycorrhizae in the interaction of phosphorus with zinc, copper and other elements. J Am Soc Soil Sci 43:976–980
Lange NR, Vlek PLG (2000) Mechanism of calcium and phosphate release from hydroxy-apatite by mycorrhizal fungi. J Am Soc Soil Sci 64:949–955
Lester D (2009) Buying and applying mycorrhizal fungi. Max. Yield, USA, pp 126–131
Mader P, Edenhofer S, Boller T, Wiemken A, Niggli U (2000) Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. J Biol Fertil Soils 31:150–156
McNeil AM, Wood M (1990) Fixation and transfer of nitrogen from white clover to ryegrass. Soil Use Manag 6:84–86
Menendez AB, Scervino JM, Godeas AM (2001) Arbuscular mycorrhizal population associated with natural and cultivated vegetation on a site of Buenos Aires Province, Argentina. J Biol Fertil Soils 33:373–381
Mohammad A, Khan AG, Kueck C (2000) Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi. Mycorrhiza 9:337–339
Morandi D (1996) Occurrence of phytoalexins and phenolic compounds in endomycorrhizal interactions and their potential role in biological control. Plant Soil 185:241–251
Morton JB (1985) Variation in mycorrhizal and spore morphology of *Glomus occultum* and *Glomus diaphanum* as influenced by plant host and soil environment. Mycologia 77:192–204
Morton JB (1988) Taxonomy of VA mycorrhizal fungi: classification, nomenclature, and identification. Mycotaxon 32:267–324
Mozafar A, Anken T, Ruh R, Frossard E (2000) Tillage intensity, mycorrhizal and nonmycorrhizal fungi and nutrient concentrations in maize, wheat and canola. J Agron 92:1117–1124
Mridha MAU, Xu HL (2001) Nature farming with vesicular-arbuscular mycorrhizae in Bangladesh. J Crop Prod 3:303–312
Murashige T, Skoog F (1962) revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15:473–497
Newman EI (1988) Mycorrhizal links between plants: their functioning and ecological significance. Adv Ecol Res 18:243–270
Newman EI, Ritz K (1986) Evidence on the pathways of phosphorus transfer between vesicular-arbuscular mycorrhizal plants. New Phytol 104:77–78
Norman JR, Hooker JE (2000) Sporulation of *Phytophthora fragaria* shows greater stimulation by exudates of non-mycorrhizal than by mycorrhizal strawberry roots. Mycol Res 104:1069–1073
Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemken A (2003) Impact of land use intensity on the species diversity of mycorrhizain agroecosystems of Central Europe. Appl Environ Microbiol 69:2816–2824
Ozaki A, Rayns FW, Gosling P, Bending GD, Turner MK (2004) Does organic farming favour arbuscular mycorrhizal fungi. In: Proceedings of the BGS/AAB/COR conference, 20–22 April, Harper Adams University College, Newport, pp 260–262
Pacovsky RS (1986) Micronutrient uptake and distribution in mycorrhizal or phosphorus-fertilized soybeans. Plant Soil 95:379–388

Pattanayak SK, Sureshkumar P, Tarafdar JC (2009) New vista in phosphorus research. J Indian Soc Soil Sci 57:536–545

Phillips JM, Hayman DS (1970) Improved procedures for clearing and staining parasitic and vesicular mycorrhiza for rapid assessment of infection. Trans Brit Mycol Soc 13:31–32

Pond EC, Menge JA, Jarrell WM (1984) Improved growth of tomato in salinized soil by VAM fungi collected from saline soils. Mycology 76:74–84

Quarles W (1999) Plant disease control and VAM fungi. IPM Pract 21:1–9

Ravnskov S, Larsen J, Jakobsen I (2002) Phosphorus uptake of an arbuscular mycorrhizal fungus is not affected by the biocontrol bacterium *Burkholderia cepacia*. J Soil Biol Biochem 34:1875–1881

Read DJ, Francis R, Finlay RD (1985) Mycorrhizal mycelia and nutrient cycling in plant communities. In: Fitter AH (ed) Ecological interactions in soil. Oxford Blackwell Scientific, London, pp 193–217

Rillig MC (2004) Arbuscular mycorrhizae, glomalin and soil aggregation. Can J Soil Sci 84:355–363

Rupam K, Deepika S, Bhatnagar AK (2008) Arbuscular mycorrhizae in micropropagation system and their applications. Sci Hortic 116:227–239

Ryan MH, Graham JH (2002) Is there a role for mycorrhiza in production agriculture. Plant Soil 244:263–271

Ryan MH, Chilvers GA, Dumaresq DC (1994) Colonization of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant Soil 160:33–40

Scholten LC, Timmermans CWM (1992) Natural radioactivity in phosphate fertilizers. Nutr Cycl Agroecosyst 43:103–107

Shafi A, Mahmood I, Siddiqui ZA (2002) Integrated management of root-knot nematode *Meloidogyne incognita* on chickpea. Thai J Agric Sci 35:273–280

Sharma S, Dohroo NP (1996) Vesicular-arbuscular mycorrhizae in plant health and disease management. Int J Trop Plant Dis 14:147–155

Shrestha-Vaidya G, Shrestha K, Khadge BR, Johnson NC, Wallander H (2008) Organic matter stimulates mycorrhizain *Bauhinia purpurea* and *Leucaena diversifolia* plantations on eroded slopes in Nepal. Restoration Ecol 16:79–87

Siddiqui ZA, Mahmood I (1995a) Role of plant symbionts in nematode management: a review. Bioresour Technol 54:217–226

Siddiqui ZA, Mahmood I (1995b) Some observations on the management of the wilt disease complex of pigeonpea by treatment with a vesicular arbuscular fungus and biocontrol agents for nematodes. Bioresour Technol 54:227–230

Siddiqui ZA, Mahmood I (1996) Biological control of *Heterodera cajani* and *Fusarium udum* on pigeonpea by *Glomus mosseae*, *Trichoderma harzianum* and *Verticillium chlamydosporum*. Israel J Plant Sci 44:49–56

Siddiqui ZA, Mahmood I (2001) Effects of rhizobacteria and root symbionts on the reproduction of *Meloidogyne javanica* and growth of chickpea. Bioresour Technol 79:41–45

Siddiqui ZA, Mahmood I, Hayat S (1998) Biocontrol of *Heterodera cajani* and *Fusarium udum* on pigeonpea using *Glomus mosseae*, *Paeciliomyces lilacinus* and *Pseudomonas fluorescens*. Thai J Agric Sci 31:310–321

Singh G, Tilak KUBR (2001) Techniques of AM fungus inoculum production. In: Mukerji KG, Manoharachary C, Chamola BP (eds) Techniques in mycorrhizal studies. Kluwer Academic Publishers, The Netherlands, pp 273–283

Smith SE, Read DJ (1997) Vesicular-arbuscular mycorrhizas in agriculture and horticulture. In: Smith SE, Read DJ (eds) Mycorrhizal symbiosis, 2nd edn. Academic Press, London, pp 453–469

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London. ISBN-10:0123705266

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1995) Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an in vitro dual culture system with the vesicular arbuscular

mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5:431–438

St-Arnaud M, Hamel B, Vimard B, Caron M, Fortin JA (1997) Inhibition of *Fusarium oxysporum F. 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

Stockdale EA, Lampkin NH, Hovi M, Keatinge R, Lennartsson EKM et al (2001) Agronomic and environmental implications of organic farming systems. Adv Agron 70:261–262

Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. Physiol Plant 102:285–296

Subramanian KS, Charest C (1999) Acquisition of external hyphae of an arbuscular mycorrhizal fungus (*Glomus intraradices* Schenck & Smith) and its impact on physiological responses in maize (*Zea mays* L.) under drought-stressed and well watered conditions. Mycorrhiza 9:69–75

Sylvia DM, Will ME (1988) Establishment of vesicular-mycorrhiza and other microorganisms on beach replenishment site in Florida. Appl Environ Microbiol 54:348–352

Takahashi S, Anwar MR (2007) Wheat grain yield, phosphorus uptake and soil phosphorus fraction after 23 years of annual fertilizer application to an Andisol. Field Crops Res 101:160–171

Thingstrup I, Rubaek G, Sibbesen E, Jakobsen I (1998) Flax (*Linum usitatissimum* L.) depends on mycorrhiza for growth and P uptake at intermediate but not high soil P levels in the field. Plant Soil 203:37–46

Van der Heijden MGA, Klironomos M, Ursic P, Moutoglis, Streitwolf-Engel R et al (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Van der Heijden MGA, Rinaudo V, Verbruggen E, Scherrer C, Barberi P, Giovannetti M (2008) The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. In: Proceedings of the 16th IFOAM Organic World Congress, 16–20 June, Modena, Italy, pp 1–4

Vassilev N, Nikolaeva I, Vassileva M (2005) Polymer based preparation of soil inoculants: applications to arbuscular mycorrhizal fungi. Rev Environ Sci Biotechnol 4:235–243

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Watson CA, Atkinson D, Gosling P, Jackson LR, Rayns FW (2002) Managing soil fertility in organic farming systems. Soil Use Manag 18:239–247

Weissenhorn I, Leyval C, Berthelin J (1993) Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy metal polluted soils. Plant Soil 157:247–256

Whipps M (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

White JA, Munn JC, William SEW (1989) Edaphic and reclamation aspects of vesicular-arbuscular mycorrhizae in Wyoming red desert soils. J Soil Sci Soc Am 53:86–90

Zou X, Binkley D, Doxtader KG (1992) A new method for estimating gross phosphorus mineralization rate in soils. Plant Soil 147:243–250

Chapter 22
RETRACTED CHAPTER: Arbuscular Mycorrhizal Fungi: A Potential Tool for Restoration of Degraded Land

Razia Shuab, Rafiq Lone, Javaid Ahmad, and Zafar A. Reshi

Abstract Arbuscular mycorrhizal fungi (AMF) have mutualistic relationships with most of the terrestrial plant species. AMF symbiosis is well known in helping the land plants to adapt to different biotic and abiotic conditions for better survival, growth and development. These symbionts offer an ecofriendly sound biological alternative to chemical fertilizers and pesticides, and hence maintaining plant quality and productivity in agriculture, horticulture and forestry. Thus, agriculturalists and soil scientists must pay proper attention to utilization of AMF to enhance, restore or maintain soil fertility and plant growth. Worldwide experiences reveal that restoration and restitution projects of degraded lands achieve little success or even fail. It is in this perspective that studies have shown that AMF can play a key role in the restoration of degraded ecosystems through beneficial impacts on plant growth and soil quality. Here we review the current knowledge and understanding about the role of AMF in improvement of soil characteristics, above- and below-ground biodiversity, seedling survival, growth and establishment particularly under stressful conditions. Developing widely accepted cost effective methods of inocula production and *in situ* AMF management for effective restoration of degraded lands shall remain the major research focus in view widespread degradation of habitats due to various anthropogenic activities.

This chapter has been retracted by editors due to a significant overlap with a previously published article by Asmelash et al. The retraction note to this chapter is available at https://doi.org/10.1007/978-3-319-68867-1_27

Shuab • Z.A. Reshi
Department of Botany, University of Kashmir, Srinagar, Jammu and Kashmir, India

R. Lone (✉)
Department of Botany, SBBS University, Khiala, Jalandhar, Punjab 144030, India
e-mail: rafiqlone@gmail.com

J. Ahmad
Government Model Science College, Jiwaji University, Gwalior, Madhya Pradesh 474011, India

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_22

22.1 Introduction

Arbuscular mycorrhiza fungi is regarded as the most ancient and widespread type of mycorrhiza (Smith and Read 2008). It is the mutualistic symbiosis between soil borne fungi with the roots of higher plants (Sieverding 1991) and the term mycorrhiza was coined by German forest pathologist Frank in (1885) and is derived from the combination of two words one Greek *mykes* (mushroom/fungus) and other Latin *rhiza* (roots), literally meaning fungus-root (Allen 1991). Mycorrhizal associations involve three-way interactions between host plants, mutualistic fungi and soil factors and now many bacterial strains have been reported to be able to promote either arbuscular or ectomycorrhizal symbiosis (Garbaye 1994; Barea et al. 2002a, b, c; Johansson et al. 2004; Artursson et al. 2006; Duponnois 2006; Frey-Klett et al. 2007; Rigamonte et al. 2010). Mycorrhiza is considered as fundamental part of the plant as 95% of all the plant families are predominantly mycorrhizal (Remy et al. 1994). Except for some angiospermic families viz., Brassicaceae, Zygophyllaceae (Verma 1998) Proteaceae (Nicolson 1967; Brundrett et al. 1996), Cactaceae, Chenopodiaceae, Cyperaceae, Amaranthaceae and Juncaceae (Hirrel et al. 1978), Dipterocaceae, Betulaceae, Myrtaceae, Fagaceae (Nicolson 1967), all others show mycorrhizal association. The mycorrhizal symbiosis is a keystone to the productivity and diversity of plants and it is rare to find a situation where AMF do not have a significant ecological importance. The perturbation or loss of this relationship can have serious consequences in terms of plant health and productivity. Available fossil and molecular evidences support the concept that this symbiosis is of ancient origin and dates back to 450 million years, implying a very long period of co-evolution of plants and fungi (Remy et al. 1994).

Most of the research efforts are concerned with mycorrhiza as a mutualistic association between the underground root of the host and soil fungi. However, there are some reports that besides roots, these fungi can also associate mutualistically with underground stem modifications like rhizomes, and other associated structures. Taber and Hepper (1982) reported for the first time, the presence of AMF in the vascular system of rhizomatous tissue and the scale like leaves of *Zingiber officinale* L. Later Nazim (1990) reviewed the presence of AMF associated with the portions other than roots in twenty one angiosperms and some non-angiosperm species. Incidence of AMF colonization has been reported in scale leaves and leaf bases of *Curcuma longa* L. (Sampath and Sullia 1992), corms of *Amorphophallus commutatus* Engler (Rodrigues 1995) and tubers of *Pueraria tuberosa* (Rodrigues 1996). Arbuscular mycorrhizal fungi have been documented in tubers of *Colocasia esculenta* (Bhat and Kaveriappa 1997), garlic bulbs (Kunwar et al. 1999) tubers of *Gloriosa superba* L. (Khade and Rodrigues 2003) and scales of *Crocus sativus* (Lone et al. 2016).

Present review emphasizes the mycorrhizal symbiosis as a keystone to plant productivity and diversity because of their influence on almost all metabolic processes of plants. AMF symbiosis hence have remarkable role in sustainable growth and development of plants.

22.2 Arbuscular Mycorrhizal Fungi (AMF)

The molecular phylogeny and fossil evidences suggest that arbuscular mycorrhizal fungi are widespread in ecosystems and very ancient in origin (Simon et al. 1993; Remy et al. 1994; Redecker 2000). The fossil records of AMF symbiosis dates back to the Ordovician age, 460 million years ago (Redecker 2000). These fossils indicate that *Glomeromycota* like fungi may have played a critical role in facilitating the colonization of land by plants. The discovery of arbuscules in Aglaophyton major, an early Devonian plant provides unequivocal evidence that mycorrhizae were established more than 400 million years ago (Simon et al. 1993; Taylor et al. 1993; Remy et al. 1994). They are believed to have been instrumental in the colonization of land by plants during that time (Remy et al. 1994; David-Schwartz et al. 2003). Recently reclassified as a part of monophyletic phylum of fungi—the *Glomeromycota* (Schubler et al. 2001).

The co-evolution of plants and AMF seem to be the key factors in the evolution of the first rootless plants to colonize the land (Pirozynski and Malloch 1975; Simon et al. 1993; Schubler 2002). Arbuscular mycorrhizal fungi have been colonizing many species of simple thalloid liverworts in Metzgeriales (*Pellia* and *Fossombrionia*), calobryales (*Hoplomitrium*) as well as complex thalloid species in the marchantials (Boullard 1988; Read et al. 2000; Carafa et al. 2003; Russell and Bulman 2005). These AMF, thus in general have helped the land plants to acclimatize the biotic and abiotic conditions for their better survival, growth and development.

Arbuscular Mycorrhizal (AM) fungi alleviates nutrient and drought resistance there by stabilizing the soil and improve plant growth and development. The Importance of AMF and their contributions towards the field of agriculture are well-known. Usually, for the restoration and recovery of the degraded areas, first step recommended is an evaluation of the mycorrhizal status of degraded land. AMF strengthens both physically as well as chemically, the soil structure. Physically the soil particles are interlinked to each other and also to the host plant through the hyphal network of the associated AMF. Chemically, a sticky substance called as glomalin is produced by AM fungi that is important in soil aggregation Wright and Upadhyaya (1998). Naturally glomalin produced by the fungi binds soil aggregates together while still allowing the free movement of nutrients, water and soil fauna within the soil (St. John 1998). Bashan et al. (2000) conducted experiments for evaluating importance AMF inoculum for establishing of six species of cactus under native mesquite (*P. articulata*) trees, and the results suggested that AM fungal inoculum improves potential in these hot desert soils. However, it was also concluded that the basic factor for the establishment of these three species is not the AM fungal inoculum density alone but favorable edaphic factors probably play a more crucial role.

Restoration ecology even before emerging as a separate discipline, the vital role of AMF in ecological restoration was been well recognized (Janos 1980). However, as of yet, there is no report available to confirm that AMF inoculation has grown to be a biotechnological tool that is widely applicable in ecological restoration.

The positive effects of AMF on associated plants' drought resistance can enhance plants' salinity tolerance as well. Improved uptake of water in plants can promote the dilution of salts within the plant cells (Larcher 1995). Resistance to plant pathogens and herbivory gets promoted via AMF. Gianinazzi et al. (2010) has described AMF as 'health insurance' for plants on considering its role in bio protection. Having synergistic interaction of AMF with plant growth promoting rhizobacteria (PGPR) is an underlying mechanism through which AMF increase plants' pathogen tolerance. A well-established role is performed by PGPR in plant pathogen inhibition (Figueiredo et al. 2010). Explanation for the role of AMF in herbivory inhibition may also be generated from the fat that AMF stimulates the synthesis of secondary metabolites (Gianinazzi et al. 2010) in plants. Apart from this, compensatory growth is the other reason through which AMF enhances the tolerance of pant against herbivory. A microcosm investigation showed that AMF associated plants despite having leaves fed by the grasshoppers, did not show any reduction in the total above ground biomass indicating that even after herbivore attack, mycorrhiza aided the plant to compensate its growth (Kula et al. 2005). AMF helps in increasing the productivity, field survival, tree/shrub Seedlings Growth and establishment on degraded lands. Lekberg and Koide (2005) carried out a meta-analysis for determining the role played by AMF for the plant growth and productivity and also the effects of three common AMF management methods; short fallow, inoculation, and reduced soil disturbance were determined. The result of the meta-analysis showed that AMF generally enhances growth and productivity of a plant grown alone. Lin et al. 2015 conducted a recent meta-analysis concluded that AMF inoculation in individual plants improves the growth and productivity. Similar result was also reported by Kirhane et al. (2014). Huante et al. (2012) also from his experiment on six tree species showed that AMF inoculation has good effect on the growth of seedlings growth and more significantly on slow growing tree species. One of the important factor that affects the restoration process of degraded areas is the tree survival and field establishment. So, AMF are vital, as they can significantly improve the field survival and also the establishment of tree seedlings. In this regard, Pouyu-Rojas and Siqueira (2000), Habte et al. (2001), Kapulnik et al. (2010), Karthikeyan and Krishnakumar (2012), and Manaut et al. (2015) have demonstrated the positive effect AMF. For the effect of the AMF on several tree species seedlings, Pouyu-Rojas and Siqueira (2000) investigated their survival and establishment on degraded pot soils. They found out a significant positive effect of AMF inoculation during transplanting or in the nursery on trees survival and establishment. Later Habte et al. (2001) determined the effect of AMF nursery inoculation has on field establishment of *Acacia koa* and accordingly, it was found that AMF improved establishment of the transplanted tree seedlings and also their growth by increasing seedlings phosphorus nutrition. The role of native AMF inoculation was also demonstrated to have a significant positive effect on the field survival and establishment of *Cupressus atlantica* Gaussen seedlings on a degraded Moroccan field site (Ouahmane et al. 2006). Similarly, Kapulnik et al. (2010) determined AMF nursery inoculation effect on seedlings field establishment and growth of *Olea europaea* L. Meanwhile, they were able to observe that AMF

inoculation improved seedlings field performance significantly and most importantly for the first 2.5 years from transplanting. They also observed that AMF effect decreased with increasing seedlings age. Karthikeyan and Krishnakumar (2012) also determined AMF effect on survival and establishment of *Eucalyptus tereticornis* Sm. on pot soil of highly degraded origin (mine spoils). Meanwhile, they were able to observe that AMF inoculation almost doubled seedling survival and significantly increased establishment. Recently, Manaut et al. (2015) demonstrated that native AMF consortia inoculation of *Ceratonia siliqua* L. seedlings more than doubled seedlings survival and significantly improved seedling's height and collar diameter.

22.3 Arbuscular Mycorrhizal Fungi and Pedogenesis

Arbuscular mycorrhizal fungal hyphae play an important role in stabilization of soil aggregates by increasing the soil nutrient uptake by plant (Miller and Jastrow 1990; Hodge 2000; Hodge et al. 2001; Wilson et al. 2009). The exudation of extracellular polysaccharides and glomalin help in entangling soil particles within the hyphae network (Treseder and Turner 2007; Rillig et al. 2014). Glomalin a polysaccharide has ability to envisage carbon, which aides in formation of organic matter, binding it to silt, sand and clay particle which is described as a major factor in the formation of soil aggregates (Miller and Jastrow 1990; Bossuyt et al. 2001; Sharma et al. 2017). The Arbuscular mycorrhizal fungi association is also reported to provide, high soil temperature, soil reaction and transplant shock (Bagyaraj and Varma 1995), rehabilitation of degraded land, reclamation and enrich soil fertility (Charles et al. 2006).

Arbuscular mycorrhizal fungi in tropical regions play a crucial role for growth, survival and development of plant species and influences plant secondary succession and community structure (Janos 1996). Their benefits may involve better access to soil resources and enhancement of soil aggregation, stability and protection against phytopathogens (Newsham et al. 1995; Rillig and Mummey 2006); influences plant biodiversity and sustainability of terrestrial ecosystems (Van der Heijden et al. 1998). Ubiquitous presence of AMF and their taxonomic, genetic and functional diversity are directly related to plant and soil processes and therefore, there is an increasing interest in the assessment of the biodiversity and functions of AMF communities (Bever et al. 1996; Oehl et al. 2003; Lovelock and Ewell 2005).

Arbuscular mycorrhizal fungi, therefore, enhance plant nutrient uptake. These also stabilize soil aggregates, prevent soil erosion, enhance soil nutrient value and fertility which are prime facts for secondary succession and plant community structure.

22.4 Land Degradation

During recent years, most of the countries especially the rapidly developing nations like China are facing severe environmental issues posed by different environmental problems like land degradation and desertification (Jiang et al. 2006). Total degraded land (2010–2011) in India is 120.40 m. ha (http://www.moef.nic.in) Such issues ultimately bring changes in the regional environment and pose a great threat to the livelihood of vast native population (Yoshino 2001).

From recent surveys, it is evident that vast areas of about 90% of these grasslands are degraded to several extents (Wu and Loucks 1992). Approximately one-third of present grassland areas in China are degraded. For this degradation, numbers of causal factors have been attributed; among which overgrazing has been the most important casual factor (Green 1989; Zhou et al. 2002; Christensen et al. 2004; Zhou et al. 2005). Overgrazing by changing the primary production, nutrient cycling, organic matter decomposition, degradation and also by altering competitive relationships among plant species significantly affects the community dynamics (McNaughton 1985; Fahnestock and Detling 1999). In soils, AMF constitutes largest component (spores and mycelia) of the microbial biomass (Miller et al. 1995). Arbuscular Mycorrhizal Fungi perform a vital ecological role in the terrestrial ecosystem in deciding plant diversity along with specie composition (van der Heijden et al. 1998). Environmental factors like soil nutrient contents and land use (Landis et al. 2004; Hijri et al. 2006) in addition to plant diversity (Eom et al. 2001; Börstler et al. 2006) have a huge impact on the AMF composition. Hence several grassland management practices like mowing, restoration, fertilization or grazing may affect the colonisation and diversity of AMF (Bethlenfalvay et al. 1985; Eom et al. 2001; Börstler et al. 2006).

Restoration is a well-defined strategy utilized for recompensing the degraded land to its native state. Restoration practices like making increments in the soil properties and enhancing vegetation cover may be encouraging methods for the reclamation of the soil productivity together with sustainability (Cooke and Johnson 2002). Ecosystem functioning may get modified via land restoration, which brings up certain biological changes like alterations in the microbial biomass and organic matter decomposition (Potthoff et al. 2006). With the help of microorganisms, debarred lands can be restored to a larger extent for achieving a well stable, pollution free, visual improvement and ultimately removal of threats to humans.

The basic act of soil disturbance, due to the destruction of mycorrhizal fungal network in soil system and its reestablishment is a vital approach of habitat restoration. By application of "biological tools" like mycorrhizal fungi inoculated tree seedlings, shrubs, and grasses, a favourable revegetation of severely disturbed mine lands can be achieved. In this regard, microbial inoculants can assist plants to manage in inimical conditions, arbuscular mycorrhizal (AM) fungi have an amazing importance as they increase nutrient procurement by the plant as well as resistance to both biotic and abiotic stress (Barea and Jeffries 1995; Barea et al. 2002a, b, c). In fact, AM fungi associations has been proposed as one of the

mechanisms of heavy metal plant tolerance (Hildebrandt et al. 2007) and water stress avoidance (Augé 2004; Ruiz-Lozano and Azcón 1995; Ruiz-Lozano et al. 1996). Generally poor soil structure, low organic matter, nutrient deficiency and low water-holding capacity are the characteristics of these arid soils. Thus, mycorrhizal inoculation alone may not be sufficient to soothe the establishment of plant cover.

Therefore, for carrying out successful reforestation, it is important to improve soil quality and the capability of the plant species for facing harsh environments. In this regard, prior to the inoculation of AM fungi, the application of organic amendments to the soil has been recommended (Medina et al. 2004). The beneficial effects of organic amendments include provision of plant nutrients, increase humus content and thereby improved soil structure, increased water-holding capacity and increased microbiology activity (Caravaca et al. 2002).

Restoration of the ecological systems has been the main theme of global environmental policies (Aradottir and Hagen 2013; Jacobs et al. 2015). Restoration of at least 15% of the world's degraded ecosystems is UN Convention on Biological Diversity (CBD 2010). In 2011 "Bonn challenge", a global commitment was endorsed by the world leaders in order to restore 150 million hectares of deforested and degraded lands by the year 2020 (Aradottir and Hagen 2013). Furthermore, in 2014 a much bigger global commitment for restoration of 350 million hectares of deforested and degraded lands until 2030 was put forward by the New York Declaration on Forests (Jacobs et al. 2015). On the basis of priority these global commitments, in 2015, were concretized by the UN by adopting the 2030 Sustainable Development Goals that constitutes 17 targets, of which the Target 15 deals with ecological restoration (UN 2015). But so far restoration experiences globally show that most of the projects granted for the restoration purposes have shown no or little effect in achieving little success or have completely failed (Thomas et al. 2014). So, a well-planned strategy and more efforts have to be employed for achieving the goals of global restoration commitments put forth. Regarding this, we propose AMF inoculation and in situ management can be better restoration outcome of degraded and deforested lands. Nearly, 93% of the families of flowering plants (Brundrett 2009) and about 92% of families comprising land plants (Wang and Qiu 2006) are known to possess mycorrhizal associations. Evolutionarily being ancestor of all the association types, arbuscular mycorrhiza is the most predominant (Wang and Qiu 2006).

For the reclamation and recovery of degraded ecosystems, mycorrhizae may play a vital role. For this reclamation, number of studies carried out by different scientists suggest that through mycorrhizal or the manipulation of their indigenous populations of the degraded ecosystems, the process of recovery can be highly accelerated (Reeves et al. 1979; Janos 1980; Allen 1991). Generally, AMF propagules remain confined to upper most few centimeters of soil and reached their highest concentrations in the rhizosphere (Schwab and Reeves 1981; Bellgard 1993). When soil is partially removed or is disturbed, the number of mycorrhizal propagules significantly decrease and in general, the plants which occupy disturbed areas are non-mycotrophic (Miller 1979; Reeves et al. 1979; Janos 1980). If in a

disturbed area, a non-mycotrophic community gets established and establishment of AMF propagules is slow, retardness in the process of successional process can occur leading to serious effects on the recovery of the damaged site. In general, mycorrhizae are required by the plants from mature ecosystems for their better growth and development (obligatory mycotrophs) (Janos 1980; Brundrett 1991). Thus, recovery of the degraded areas where mycorrhizal propagules have been loss in good number, can only be attained by the reintroduction of these propagules either via anthropogenic activities or through natural processes.

22.5 Determinable Restoration Attributes and AMF

AMF plays a key role in the ecological restoration and recovery by making improvements in the nutrient uptake and accumulation, plant fitness (growth, survival and reproduction), diversity (richness and evenness) and succession and also that of animal communities (Direct effects on organisms which feed on fungi and indirect effects due to changes in plant fitness), tolerance of adverse conditions (biotic and abiotic stresses) and altering plant community structure (competition/facilitation) (Brundrett and Abbott 2002). Based on Aronson et al. (1993), for the improvement of the ecological restoration, the structural and functional characteristics includes; plant and microbial diversity as well as abundance, soil organic matter, index of nutrient cycling, plant productivity and soil water relation. Key role of AMF regarding the functional and structural attributes are highlighted below:

22.5.1 AMF Improve Soil Aggregation

AMF increase soil organic matter and soil water relation; thereby increasing total soil organic matter as well as soil water relation for stabilizing the soil structure, the fungi especially AMF may prove as the most effective soil organisms (Augé 2004). AMF hyphae bind soil aggregates by penetrating into the soil matrix creating a skeletal structure holding the primary soil particles (Augé 2004; Al-Karaki 2013). AMF by influencing the growth of soil bacterial communities can also improve the formation of soil aggregates (Rilling 2004). Furthermore, glomalin, a hydrophobic stable aggregate former, is produced from the dead AMF hyphae (Barea et al. 2002a, b, c; Simard and Austin 2010). Thus, AMF increase and improves both soil aggregation and stability. AMF has a power to survive in soil even up to 5 months after their host's death (Soka and Ritchie 2014). Meanwhile, improved soil aggregation and stability along with significant amount of mycorrhizal derived soil carbon (Rilling 2004), AMF increases stability and soil organic matter content (Rilling 2004; Leifheit et al. 2014). Soil water relations also show improvements through soil aggregation. It was observed that in a mycorrhizal soil a naturally non-mycorrhizal grown plant tolerated drought in contrast to the ones grown in

some non-mycorrhizal soils indicating that hyphal network produced from AMF improves water holding capacity of soils (Marschner 1995).

22.5.2 Mycorrhizal Inoculation Technology for the Recovery of Degraded Land

Revegetation processes either natural or artificial in Mediterranean ecosystems can get halted, due to loss of mycorrhizal propagules following degradation of the vegetation cover. Augmentation of the inoculum potential may be required (Requena et al. 2001). In certain cases, shrubs inoculated by mycorrhiza—act as a "resource islands" (Allen 1988; Azcón-Aguilar et al. 2003; Caravaca et al. 2005), serving as a source of inoculum for the surrounding area thus stimulating revegetation. Nursery production of quality native seedlings with a balanced mycorrhizal status is a key strategy for mycorrhizal application for revegetation. Recently the biotechnological procedures followed to produce ectendomycorrhizal *Helianthemum* plants with *Terfezia* species (desert truffles) have also been discussed (Morte et al. 2009).

Regarding revegetation tactics for degraded areas, the first investigation on the importance of using native plant species inoculated by mycorrhiza was from South East Spain in its semi-arid desertification threatened environment south of the Sierra Nevada, Granada (Herrera et al. 1993). Woody legumes as plant species were used, having symbiotic association with both N_2-fixing rhizobial bacteria and AM fungi, associations which enable the plant to grow and develop in low water and nutrient deficient conditions (Azcón and Barea 2010). Four non-native tree legumes Robinia pseudo-acacia L., *Acacia caven* (Mol.) Mol. and *Prosopis chilensi* and two native shrubs (*Anthyllis cytisoides* and *Spartium junceum* L.), were target legumes. The Society for Ecological Restoration's (SER 2004) defines ecological restoration as the process of assisting in the recovery and reclamation of a degraded ecosystem. Because of the anthropogenic activities, ecosystems of the earth have been continuously disturbed (Araujo et al. 2013: Pabst et al. 2013). Nearly about 40% of the total terrestrial vegetation has been directly disturbed and the natural productive capacity has got reduced through deforestation, agriculture, overgrazing, over exploitation for fuel wood, urban and industrial use (Cooke and Johnson 2002).

22.5.3 Renovation of Degraded Lands via AMF Biotechnology

Low dense AMF along with nursery seedlings around is very likely to make its infection in the degraded sites (Michelsen 1992). Thus, such sites on the reintroduction of sufficient AMF inocula can brace the growth of delayed

successional tree species. Being obligatory mycotrophic, such late tree successional for their continuance and wellbeing inevitably depend upon AMF (Janos 1980). However, at the time of early growing stage of seedling, successional Trees/shrub species contrary to late successional ones for their growth and survival are more dependent on AMF association (Kiers et al. 2000). Thus, for the restoration and renovation of the degraded lands AMF inoculations could be taken as substantial biotechnological tool. Several researchers are of the opinion that like in mine fields AMF inoculation can be better suited only to few conditions where the native AMF inoculum has either disappeared or might be present in little (Brundrett and Abbott 2002). Koide and Mosse (2004) suggested that it would be better to concentrate on the managing of the native AMF population of a site instead of going for the AMF inoculation. According to Renker et al. (2004), AMF inoculation is highly substantial yet the last option. Nevertheless, AMF dispersal contrary of having various dispersing agents like water, wind, birds, ants, worms and rodents were quite poor (Brundrett and Abbott 2002). Likewise, Hailemariam et al. (2013) successfully made observations that AMF dispersal in short distances, within a piece of farmland can show great variations regarding soil AMF status and ineffectiveness ultimately signifying their poor dispersal. So, under such conditions AMF inoculation may be the most reliable intervention. Similarly, under the different ranges of soil conditions, AMF inoculation has been found effective (Janos 1980; Brundrett and Abbott 2002) along with soils having ample AMF (Bainard et al. 2013). Unequivocal effect of AMF associations is not certified by the abundant presence of native AMF, however abundance along with adeptness of the indigenous AMF are determinant (Onguene and Kuyper 2005). Veiga et al. (2011) also hypothesized that AMF inoculation could possibly restrain ruderal plants by muffling weeds which otherwise can invade the graded sites. This is vital for ecological restoration as ruderal plants invasion to degraded land will lead to competition for the living of planted seedlings of trees/shrubs. If the restoration of degraded lands *via* AMF inoculation is accepted, next question arising will be; what type of inocula has to be prepared? AMF display a wide range of functional diversity (Johnson et al. 1997; Klironomos 2003; Smith and Smith 2011) and their effect is within the mutualism–parasitism continuum (Johnson et al. 1997). Klironomos (2003) also demonstrated that exotic–native AMF strain-host or vice-versa combination results in highly parasitic interaction. So, decision for preparing a specific type of AMF inoculum is a key step. Based on recent available data, preference should be given to the use of indigenous inocula to that of exotic inocula. For the restoration of seedling along with even lateral seral tree species, early seral AMF should be utilized (Allen et al. 2003). Seedlings inoculated with early successional AMF having smaller pores and low carbon demand are highly beneficial contrary to late seral AMF possessing huge spores and higher carbon demand (Allen et al. 2003). Promotion for the use of the inocula from grasslands is quite higher and also abundant that can be more than tenfold contrary to forest lands (Fischer et al. 1994). Because of this Onguene and Kuyper (2005) used fresh grass land whole-soil inoculum on several soils and also on three tree species seedlings. Results obtained by Onguene and Kuyper (2005) obtained, showed that though there was a positive effect of early successional

inoculum for most near about 80% of cases, but the results also showed significant negative effect by the inoculum, from the grasslands on *Terminalia superba* Engl. and Diels seedlings growing on agricultural and early successional forest soils. So, Onguene and Kuyper (2005) concluded that all of the allochthonous AM inocula may not always show their effectiveness. Therefore, use of planting site adapted AM inocula may be recommended. Host plant's fungi specificity might be the other reason for the observed negative effect (Onguene and Kuyper 2005).

Research data on AMF show that inocula obtained from conspecific source display better affinity to associated plant root (Kiers et al. 2000). Recent available data also reveal that some of the plant species that even co-occur may show preferences for ... associations with distinct AMF communities (Wubet et al. 2006; Davison et al. 2011). From some available data of AMF, it is obvious that seedlings as well as ro... of a single tree species get colonized by different AMF communities (W... et al. 2009). Hence question arises; does the AMF inoculums derived from adu... seedlings or rhizosphere on inoculation produce an impressive effect? Kie... et al. (2000) have found that though seedlings inoculated by the inoculum from adults possessed good affinity but its effect on growth was marginally low, displaying that seedlings inoculated even by the inocula from may not show an impr... impact. Increasing the density of few of the dominant AMF species and applying... inocula had resulted in negative effects on plant growth by disrupting in... genous AMF community structure and thereby creating competition among AMF to u... ately result in inoculum failure (Janoušková et al. 2013). Therefore, in are... with low levels of indigenous AMF abundance, multiplying all not only the d...inant AMF species and applying all may be the best option. The AMF richness in AM... inocula is considered to improve inocula effectiveness. Plant response is sub...ntially lower when inoculated with single AMF species and the response keep... increasing from multiple fungal species to whole-soil inoculums (Hoeksema et al. 201... Likewise, Barea et al. (2011) compiling long years of experience in AMF research recommend the use of autochthonous foundation shrub inoculated with autoc...honous AMF consortia inoculums to best restore degraded lands of the Mediterranean. The shrub not only acts as a foundation species but also serves as a resource island for AMF (Barea et al. 2011). However, not all ecologists agree by the ...plication of AMF species rich inocula; some argue that better results due to inoc...la... better AMF species richness is due to sampling effect and selecting sing... effect... AMF species should get the attention of restoration ecologists.

Th... other challenge associated with AMF biotechnology is related with inocula production for large-scale application. This is due mainly to the obligate nature of AMF. Meanwhile, AMF cannot be cultured axenically (Azcón-Aguilar et al. 1999; F...tin et al. 2005) and host plant based AMF multiplication is mandatory. These host plant based conventional inocula production methods (substrate based pot culturing and substrate free methods of hydroponic and aeroponic techniques) are costly and large scale production of AMF inocula may hardly be possible. Effective monexenic in vitro culturing of AMF has been made possible few decades ago (Bécard and Fortin 1988) and in India, using this method, large-scale industrial production of biologically clean AMF inocula was possible (Adholeya et al. 2005). Adholeya et al. (2005) and Cranenbrouck et al. (2005) developed technique of

monoxenic in vitro AMF culture production for large-scale application. However, until now, monoxenic in vitro culturing is not widely practiced. This is due mainly to the fact that; (I) undesired contamination is hardly avoidable and the technique is technology and skill demanding (Bago and Cano 2005), (II) there are ethical and legal concerns, and (III) it is rather very hard to identify each genotype (even morphotype) hence, most if not all, AMF are not readily culturable (Fortin et al. 2005). AMF monoxenic in vitro culturing uses transformed roots as host owing to the fact that these hairy roots are better suited than the non-transformed hairy roots since they grow on hormone free media and without developing shoots and leaves (Puri and Adholeya 2013). Meanwhile, AMF monoxenic culture as it is practiced now could potentially be challenged with biosafety related issues.

Due to the lack of cheap and easy AMF inocula production for large scale application, managing the in situ AMF is sometimes considered to be an effective AMF biotechnology for the restoration of degraded lands. The meta-analysis by Lekberg and Koide (2005) showed that short fallow could be a good as inoculation to improve plants growth and productivity. It was shown that an obligatory arbuscular mycorrhizal pioneer nurse shrub *Lavandula stoechas* L. improved the field survival and establishment of *Cupressus atlantica* Gaussen seedlings by increasing, among others, in situ infective AMF abundance (Duponnois et al. 2011). Kumar et al. (2010) also compared different plant composition effects on in situ management of AMF on a degraded coal mine spoil. Accordingly, they demonstrated that using cover crops mainly grasses and N-fixing shrubs in the plant composition, significantly enhanced AMF abundance, diversity and infectiveness. Hence, AMF can be manipulated by fallowing or/and by designing the plant species composition to ultimately result in increased AMF abundance which in turn facilitates restoration. However, some investigations indicated that grass cover can significantly suppress individual tree/shrub seedlings-saplings growth (Riginos 2009) or may have variable seasonal effects (Good et al. 2014). Therefore, investigation on cover plant management options to effectively manage AMF and facilitate tree/shrub seedlings growth can be an important research topic. Nowadays, substrate free inocula preparation methods and in vitro production on excised plant roots are being intensively researched to make AMF inoculation less costly (Ijdo et al. 2011). The pot culture inocula preparation method, although its labor intensive and costly, can be a source of employment especially in developing countries. Therefore, pot culture based AMF biotechnology will remain to be a feasible way of degraded lands restoration in most parts of the world.

22.6 Conclusion

This review paper has compiled facts regarding the AMF role in the above and belowground ecosystem processes relevant to ecological restoration. Accordingly, it is possible to conclude that AMF; have a well-documented positive role in nutrient cycling and improved soil attributes. AMF also improve plants 'tolerance

to biotic and abiotic stresses, and significantly increase tree/shrub seedlings survival, establishment and growth. At plant community level, AMF increase both above and below ground biodiversity but their effect on primary productivity may be low. Future researches should focus on forest communities of both the temperate and tropics. For an effective large scale application of AMF inocula biotechnology, pot based inocula multiplication will remain to be significantly cost ineffective. Therefore, investigating and researching on cost effective multiplication methods of substrate free and in vitro culture and/or optimization of the effects of low-cost fresh AMF inoculation techniques like using grassland top soil or managing AMF in situ using several cover crops including grasses need further attention in the future. Optimization of monoxenic *in vitro* AMF culture products and using non-transformed hairy root organ could also be an important research area until xenic in vitro AMF culturing is ultimately made possible.

References

Adholeya A, Tiwari P, Singh R (2005) Large scale inoculum production of arbuscular mycorrhizal fungi on root organs and inoculation strategies. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 315–338

Al-Karaki GN (2013) The role of mycorrhiza in the reclamation of degraded lands in arid environments. In: Shahid SA, Taha FK, Abdelfattah MA (eds) Developments in soil classification, land use planning and policy implications: innovative thinking of soil inventory for land use planning and management of land resources. Springer Science Business Media, Dordrecht, pp 823–836

Allen MF (1988) Belowground structure: a key to reconstructing a productive arid ecosystem. In: Allen E (ed) Reconstruction of disturbed arid ecosystems. Westview Press, Boulder, CO, p 113e135

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen EB, Allen MF, Egerton-Warburton L, Corkidi L, Gomez-Pompa A (2003) Impacts of early and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. Ecol Appl 13:1701–1717

Aradottir AL, Hagen D (2013) Ecological restoration: approaches and impacts on vegetation, soils and society. Adv Agron 120:73–222. https://doi.org/10.1016/b978-0-12-407686-0.00003-8

Araujo ASF, Cesarz S, Leite LFC, Borges CD, Tsai SM, Eisenhauer N (2013) Soil microbial properties and temporal stability in degraded and restored lands of northeast Brazil. Soil Biol Biochem 66:175–181

Aronson J, Fled C, Le Floc'h E, Ode C, Pontanier R (1993) Restoration and rehabilitation of degraded ecosystems in arid and semi-arid lands: a view from the south. Restor Ecol 1:8–17. https://doi.org/10.1111/j.1526-100X.1993.tb00004.x

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

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Azcón R, Barea JM (2010) Mycorrhizosphere interactions for legume improvement. In: Khanf MS, Zaidi A, Musarrat J (eds) Microbes for legume improvement. Springer, Vienna, pp 237–271

Azcón-Aguilar C, Bago B, Barea JM (1999) Saprophytic growth of arbuscular mycorrhizal fungi. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, New York, pp 391–407

Azcón-Aguilar C, Palenzuela J, Roldan A, Bautista S, Vallejo R, Barea JM (2003) Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrub lands. Appl Soil Ecol 22:29e37

Bago B, Cano C (2005) Breaking myths on arbuscular mycorrhiza as in vitro biology. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 111–138

Bagyaraj DJ, Varma AK (1995) Interaction between VA mycorrhizal fungi and plants, and their importance in sustainable agriculture in arid and semi arid tropics. Adv Microb Ecol 14:119–142

Banerjee K, Gadani MH, Srivastava KK, Verma N, Jasrai YT, Jain NK (2013) Screening of efficient arbuscular mycorrhizal fungi for *Azadirachta indica* under nursery condition: a step towards afforestation of semi-arid region of Western India. Braz J Microbiol 44:587–595

Barea JM, Jeffries P (1995) Arbuscular mycorrhizas in sustainable soil plant systems. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Heidelberg, pp 521–559

Barea JM, Palenzuela J, Azcón R, Ferrol N, Azcón-Aguilar C (2002a) Micorrizas y restauración de la cubierta vegetal en ambientes mediterráneos. In: Barea-Azcón JM, Ballester E, Luzón JM, Moleón M, Tierno JM, Travesí R (eds) Biodiversidad y Conservación de Fauna y Flora en Ambientes Mediterráneos. Granada, España, pp 83–105

Barea JM, Azcón R, Azcón-Aguilar C (2002b) Mycorrhizosphere interactions to improve plant fitness and soil quality. Anton Leeuw Int J 81:343–351

Barea JM, Gryndler M, Lemanceau P, Schüepp H, Azcón R (2005) The rhizosphere of mycorrhizal plants. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhiza technology in agriculture: from genes to bioproducts. Birkhäuser Verlag, Basel, pp 1–18

Barea JM, Palenzuela J, Cornejo P, Sánchez-Castro I, Navarro-Fernández C, Lopéz-García A, Estrada B, Azcón R, Ferrol N, Azcón-Aguilar C (2011) Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. J Arid Environ 75:1292–1301

Bashan Y, Davis EA, Carrillo-Garcia A, Linderman RG (2000) Assessment of AM Mycorrhizal inoculum potential in relation to the establishment of cactus seedlings under mesquite nursetrees in the Sonoran desert. Appl Soil Ecol 14:165–175

Bécard G, Fortin JA (1988) Early events of vesicular-arbuscular mycorrhiza formation in Ri T-DNA transformed roots. New Phytol 108:211–218. https://doi.org/10.1111/j.1469-8137.1988.tb03698.x

Bellgard SE (1993) The topsoil as the major store of propagules of vesicular–arbuscular mycorrhizal fungi in southeastern Australian sandstone soils. Mycorrhiza 3:19–24

Bethlenfalvay GJ, Evans RA, Lesperance AL (1985) Mycorrhizal colonization of crested wheatgrass as influenced by grazing. Agron J 77:233–236

Bever JD, Morton JB, Antonovics J, Schultz PA (1996) Host dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82

Bhat PR, Karyappa KM (1997) Occurrence of vesicular arbuscular mycorrhizal fungi in the tubers of *Colocasia esculanta* (L.) Schott. Mycorrhiza News 9:12–13

Birhane E, Sterck FJ, Bongers F, Kuyper TW (2014) Arbuscular mycorrhizal impacts on competitive interactions between *Acacia etbaica* and *Boswellia papyrifera* seedlings under drought stress. J Plant Ecol 1:298–308. https://doi.org/10.1093/jpe/rtt031

Börstler B, Renker C, Kahmen A, Buscot F (2006) Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. Biol Fertil Soils 42:286–298

Bossuyt H, Denef K, Six FSD, Merckx R, Paustian K (2001) Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol 16:195–208

Boullard B (1988) Observations on the coevolution of fungi with hepatitis. In: Pyrozynski KA, Howksworth DL (eds) Coevolution of fungi with plants and animals. Academic Press, London

Brundrett M (1991) Mycorrhizas in natural ecosystems. Adv Ecol Res 21:171–313

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

Brundrett MC, Abbott LK (2002) Arbuscular mycorrhiza in plant communities. In: Sivasithamparam K, Dixon KW, Barrett RL (eds) Plant conservation and biodiversity. Kluwer Academic Publishers, Dordrecht, pp 151–193

Brundrett M, Beegher N, Dell B, Groov T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monogr 32

Carafa A, Duckett JG, Ligrone R (2003) Subterranean gametophytic axes in the primitive liverwort *Haplomitrium* harbour a unique type of endophytic association with aseptate fungi. New Phytol 160:185–197

Caravaca F, Barea JM, Figueroa D, Roldán A (2002) Assessing the effectiveness of mycorrhizal inoculation and soil compost addition for enhancing reforestation with *Olea europaea* subsp. *sylvestris* through changes in soil biological and physical parameters. Appl Soil Ecol 20:107–118

Caravaca F, Alguacil MM, Barea JM, Roldán A (2005) Survival of inoculated arbuscular mycorrhizal fungi species associated with shrubs in a degraded Mediterranean ecosystem. Soil Biol Biochem 37:227e233

CBD (2010) Aichi biodiversity targets. Available via http://www.cbd.int/sp/targets/

Charles P, Raj ADS, Kiruba S (2006) Arbuscularmycorrhizal fungi in the reclamation and restoration of soil fertility. Mycorrhiza News 18:13–14

Christensen L, Coughenour MB, Ellis JE, Chen ZZ (2004) Vulnerability of the Asian typical steppe to grazing and climate change. Climate Change 63:351–358

Cooke JA, Johnson (2002) Ecological restoration of land with particular reference to the mining of metals and industrial minerals: a review of theory and practice. Environ Rev 10:41–71

Cranenbrouck S, Voets L, Bivort C, Renard L, Strullu DG, Declerck S (2005) Methodologies for in vitro cultivation of arbuscular mycorrhizal fungi with root organs. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 341–375

David-Schwart R, Gadkar V, Wininger S, Bendor R, Gallic G, Levy AA, Kapulnik Y (2003) Isolation of a premycorrhizal infection (*Pmi2*) mutant of tomato, resistant to arbuscular mycorrhizal fungal colonization. Mol Plant Microbe Interact 16:382–388

Davison J, Öpik M, Daniell TJ, Moora M, Zobel M (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. FEMS Microbiol Ecol 78:103–115

Duponnois R (2006) Bacteria helping mycorrhiza development. In: Mukerji KG, Manoharachary C, Singh J (eds) Microbial activity in the rhizosphere. Springer, Berlin, pp 297–310

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 belowground mutualism and soil microbial activity. Soil Biol Biochem 43:2160–2168

Eom AH, Wilson GWT, Hartnett DC (2001) Effect of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia 93:233–242

Fahnestock JT, Detling JK (1999) The influence of herbivory on plant cover and species composition in the Pryor Mountain Wild Horse Range, USA. Plant Ecol 144:145–157

Figueiredo MDB, Seldin L, de Araujo FF, Mariano RDR (2010) Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Microbiology monographs. Springer, Berlin, pp 21–43

Fischer CR, Janos DP, Perry DA, Liberman RG, Sollins P (1994) Mycorrhiza inoculum potentials in tropical secondary succession. Biotropica 26:369–377. https://doi.org/10.2307/2389230

Fortin JA, Declerck S, Strullu DG (2005) In vitro culture of mycorrhizas. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 3–14

Frank AB (1885) Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäum durch unterirdische Pilze. Ber Deut Bot Ges 3:128–145

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis (Tansley review, 76). New Phytol 128:197–210

Gianinazzi S, Gollotte A, Binet MN, vanTuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of Arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530

Good MK, Clarke PJ, Price JN, Reid N (2014) Seasonality and facilitation drive tree establishment in a semi-arid flood plain savanna. Oecologia 175:261–271

Green DR (1989) Rangeland restoration projects in western New South Wales. Aust Rangel J 11:110–116

Habte M, Miyasaka SC, Matsuyama DT (2001) Arbuscular mycorrhiza fungi improve early forest-tree establishment. In: Horst WJ, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer B (eds) Plant nutrition: food security and sustainability of agroecosystems through basic and applied research. Kluwer Academic Publishers, Dordrecht, pp 644–645

Hailemariam M, Birhane E, Asfaw Z, Zewdie S (2013) Arbuscular mycorrhiza association of indigenous agroforestry tree species and their infective potential with maize in the rift valley, Ethiopia. Agrofor Syst 87:1–14. https://doi.org/10.1007/s10457-013-9634-9

Herrera MA, Salamanca CP, Barea JM (1993) Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Appl Eviron Microbiol 59:129–133

Hijri I, Sykorova Z, Oehl F, Ineichen K, Mäder P, Wiemken A, Redecker D (2006) Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. Mol Ecol 15:2277–2289

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146. https://doi.org/10.1016/j.phytochem.2006.09.023

Hirrel MC, Mehravaran H, Gerdemann JW (1978) Vesicular–arbuscular mycorrhiza in Chenopodiaceae and Cruciferae: do they occur? Can J Bot 56:2813–2817

Hodge A (2000) Microbial ecology of the Arbuscular mycorrhizal. FEMS Microbiol Ecol 32:91–96

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GW, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:267–407

Huante P, Ceccon E, Orozco-Segovia A, Sánchez-Coronado ME, Acosta I, Rincón E (2012) The role of arbuscular mycorrhizal fungi on the early-stage restoration of seasonally dry tropical forest in Chamela, Mexico. Revista Árvore Viçosa-MG 36:279–289

Ijdo M, Cranenbrouck S, Declerck S (2011) Methods for large-scale production of AM fungi: past, present, and future. Mycorrhiza 21:1–16. https://doi.org/10.1007/s00572-010-0337-z

Jacobs DF, Oliet JA, Aronson J, Bolte A, Bullock JM, Donoso PJ, Landhaeusser SM, Madsen P, Peng S, Rey-Benayas JM, Weber JC (2015) Restoring forests: what constitutes success in the twenty-first century? New For 46:601–614. https://doi.org/10.1007/s11056-015-9513-5

Janos DP (1980) Mycorrhizae influence tropical succession. Biotropica 12:56–64

Janos DP (1996) Mycorrhizas, succession and rehabilitation of deforested lands in the humid tropics. In: Frankland JC, Gadd GM (eds) Fungi and environmental change. Cambridge University Press, Cambridge, pp 1–18

Janoušková M, Krak K, Wagg C, Štorchová H, Caklová P, Vosátkaa M (2013) Effects of inoculum additions in the presence of a pre-established arbuscular mycorrhizal fungal community. Appl Environ Microbiol 79:6507–6515. https://doi.org/10.1128/AEM.02135-13

Jiang G, Han X, Wu J (2006) Restoration and management of the Inner Mongolia grassland requires a sustainable strategy. Ambio 35:269–270
Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–13
Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytol 135:575–586
Kapulnik Y, Tsror L, Zipori I, Hazanovsky M, Wininger S, Dag A (2010) Effect of AMF application on growth, productivity and susceptibility to Verticillium wilt of olives grown under desert conditions. Symbiosis 52:103–111
Karthikeyan A, Krishnakumar N (2012) Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Sm. seedlings inoculated with Arbuscular mycorrhizal fungi. Ann For Res 55:207–216
Khade SW, Rodrigues BF (2003) Incidence of Arbuscular mycorrhizal colonization in tubers of *Gloriosa superba* L. Mycorrhiza News 15:14–16
Kiers ET, Lovelock CE, Krueger KL, Herre EA (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. Ecol Lett 3:106–113
Klironomos JN (2003) Variation in plant response to native and exotic mycorrhizal fungi. Ecology 84:2292–2301
Koide RT, Mosse B (2004) A history of research on arbuscular mycorrhiza. Mycorrhiza 14:145–163
Kula AAR, Hartnett DC, Wilson GWT (2005) Effects of mycorrhizal symbiosis on tall grass prairie plant–herbivore interactions. Ecol Lett 8:61–69. https://doi.org/10.1111/j.1461-0248.2004.00690.x
Kumar A, Raghuwanshi R, Upadhyay RS (2010) Arbuscular mycorrhizal technology in reclamation and revegetation of coal mine spoils and various revegetation models. Engineering 2:683–689. https://doi.org/10.4236/eng.2010.29083
Kunwar IK, Reddy PJM, Manoharachary C (1999) Occurrence and distribution of AMF associated with garlic rhizosphere soil. Mycorrhiza News 11:4–6
Landis FC, Gargas A, Givnish TJ (2004) Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas. New Phytol 164:493–504
Larcher W (1995) Physiological plant ecology. Springer, Berlin
Leifheit EF, Verbruggen E, Rillig MC (2014) Arbuscular mycorrhizal fungi reduce decomposition of woody plant litter while increasing soil aggregation. Soil Biol Biochem 81:323–328
Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204. https://doi.org/10.1111/j.1469-8137.2005.01490.x
Lin G, McCormack ML, Guo D (2015) Arbuscular mycorrhizal fungal effects on plant competition and community structure. J Ecol 103:1224–1232
Lone R, Shuab R, Koul KK (2016) AMF association and their effect on metabolite mobilization, mineral nutrition and nitrogen assimilating enzymes in Saffron (*Crocus sativus* L.) plant. J Plant Nutr 39:1852–1862
Lovelock CE, Ewell JJ (2005) Links between tree species, symbiotic fungal diversity and ecosystem functioning in simplified tropical ecosystems. New Phytol 167:219–228
Ninaut N, Sanguin H, Ouahmane L, Bressan M, Thioulouse J, Baudoin E, Hafidi M, Prin Y, Duponnois R (2015) Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. Ecol Eng 79:113–119
Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London
McNaughton SJ (1985) Ecology of a grazing ecosystem: the Serengeti. Ecol Monogr 55:259–294
Medina A, Vassilev N, Alguacil MM, Roldán A, Azcón R (2004) Increased plant growth, nutrient uptake, and soil enzymatic activities in a desertified mediterranean soil amended with treated

residues and inoculated with native mycorrhizal fungi and a plant growth promoting yeast. Soil Sci 169:260–270
Michelsen A (1992) Mycorrhiza and root nodulation in tree seedlings from five nurseries in Ethiopia and Somalia. For Ecol Manage 48:335–344. https://doi.org/10.1016/0378-1127(92)90154-2
Miller RM (1979) Some occurrences of vesicular-arbuscular mycorrhiza in natural and disturbed ecosystems of the Red Desert. Can J Bot 57:619–623
Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol Biochem 22:579–584
Miller RM, Reinhardt DR, Jastrow JD (1995) External hyphal production of vesicular–arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. Oecologia 103:17–23
Morte A, Zamora M, Gutiérrez A, Honrubia M (2009) Desert truffle cultivation in semi-arid Mediterranean areas. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (eds) Mycorrhizas functional processes and ecological impact. Springer, Berlin, Heidelberg, p 221e233
Nazim G (1990) Vesicular arbuscular mycorrhiza in portions other than roots. In: Jalali BL, Chand H (eds) Current trends in mycorrhizal research. Sankat Mochan Art Press, Hisar, pp 4–6
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
Nicolson TH (1967) Vesicular Arbuscular mycorrhiza—a universal plant symbiosis. Sci Prog 55:561–581
Oehl F, Sieverding E, Ineichen K, Mader P, Boller T, Wiemken A (2003) Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl Environ Microbiol 69:2816–2824
Onguene NA, Kuyper TW (2005) Growth response of tropical native timber species to soils with different arbuscular mycorrhizal inoculums potentials in South Cameroon Indigenous inoculum and effect of addition of grass inoculums. For Ecol Manage 210:283–290. https://doi.org/10.1016/j.foreco.2005.02.038
Ouahmane L, Hafidi M, Thioulouse J, Duponnois R, Boumezzough A (2006) Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. J Appl Microbiol 103:683–690
Pabst H, Kuhnel A, Kuzyakov Y (2013) Effect of land-use and elevation on microbial biomass and water extractable carbon in soils on Mt. Kilimanjaro ecosystems. Appl Soil Ecol 67:10–19
Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotrophism. BioSystems 6:153–164
Potthoff M, Steenwerth KL, Jackson LA, Drenovsky RE, Scow KM, Joergensen RG (2006) Soil microbial community composition as affected by restoration practices in California grassland. Soil Biol Biochem 38:1851–1860
Pouyu-Rojas E, Siqueira JO (2000) Arbuscular mycorrhizal and soil fertilization on post-transplant development to fout plants of seven forest species. Pesq Agrop Bras 35:103–114. https://doi.org/10.1590/S0100-204X2000000100013
Puri A, Padholeya A (2013) A new system using *Solanum tuberosum* for the co-cultivation of *Glomus intraradices* and its potential for mass producing spores of arbuscular mycorrhizal fungi. Symbiosis 59:87–97. https://doi.org/10.1007/s13199-012-0213
Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal association in 'lower' land plants. Philos Trans R Soc Lond Ser B 355:815–831
Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi (Glomales) within colonized roots. Mycorrhiza 10:73–80
Reeves FB, Wagner D, Moorman T, Kiel J (1979) The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. Am J Bot 66:6–13

Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci USA 91:11841–11843

Renker C, Zobel M, Öpik M, Allen MF, Allen EB, Vosátka M, Rydlova J, Buscot F (2004) Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? In: Temperton VM, Hobbs RJ, Nuttle T, Halle S (eds) Assembly rules and restoration ecology: bridging the gap between theory and practice. Island Press, Washington, DC, pp 189–229

Requena N, Pérez-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

Rigamonte TA, Pylro VS, Duarte GF (2010) The role of mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations. Braz J Microbiol 41:832–840

Riginos C (2009) Grass competition suppresses savanna tree growth across multiple demographic stages. Ecology 90:335–340

Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

Rillig MC, Wendt S, Antonovics J, Hempel S, Kohler J, Wehner J, Caruso T (2014) Interactive effects of root endophytes and Arbuscular mycorrhizal fungi on an experimental plant community. Oecologia 174:263–270

Rilling MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol Lett 7:740–754. https://doi.org/10.1111/j.1461-0248.2004.00620.x

Rodrigues BF (1995) The occurrence of VAM fungi in the corms of *Amorphophallus commutatus* Engler. Mycorrhiza News 7:5

Rodrigues BF (1996) The occurrence of VAM fungi in the tubers of *Pueraria tuberosa* (Willd.) DC. Mycorrhiza News 8:9

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:72–478

Ruiz-Lozano J, Azcón R, Palma JM (1996) Superoxide dismutase activity in arbuscular-mycorrhizal *Lactuca sativa* L. plants subjected to drought stress. New Phytol 134:327–333

Russell J, Bulman S (2005) The liverwort *Marchantia foliaceae* forms a specialized symbiosis with AM fungi in the genus *Glomus*. New Phytol 165:567–579

Sampath P, Sullia SB (1992) The occurrence of VAM fungi in the scale leaves of turmeric. Mycorrhiza News 14:5

Schubler A (2002) Molecular phylogeny, taxonomy, and evolution of *Gossiphora pyriformis* and Arbuscular mycorrhizal fungi. Plant Soil 244:75–83

Schubler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol Res 105:1413–1421

Schwab S, Reeves FB (1981) The role of endomycorrhizae in revegetation practices in the semiarid west. II. Geographical distribution of VA-mycorrhiza inoculum potential. Am J Bot 68:1293–1297

SER (Society for Ecological Restoration International Science) (SER) & Policy Working Group (2004) The SER International primer on ecological restoration. Available from http://www.ser.org. Society for Ecological Restoration International, Tucson, Arizona

Sharma M, Prasad R, Varma A, Sharma AK (2017) Glycoprotein associated with *Funneliformis coronatum, Gigaspora margarita* and *Acaulospora scrobiculata* suppress the plant pathogens in vitro. Asian J Plant Pathol. https://doi.org/10.3923/ajppaj.2017

Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. DeutcheGesellschaftfurrTechnischeZusammenarbeit, GTZ No. 224. Eschborn, p 371

Simard SW, Austin ME (2010) The role of mycorrhizas in forest soil stability with climate change. In: Simard SW (ed) Climate change and variability. In Tech Open Access, Rijeka, pp 275–302

Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69

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 ecosystems scales. Annu Rev Plant Biol 63:227–250. https://doi.org/10.1146/annurev-arplant-042110-103846

Soka G, Ritchie M (2014) Arbuscular mycorrhizal symbiosis and ecosystem processes: prospects for future research in tropical soils. Open J Ecol 4:11–22. https://doi.org/10.4236/oje.2014.41002

St. John T (1998) Mycorrhizal inoculation in habitat restoration. Land Water 42:17–19

Taber RA, Trappe JM (1982) Vesicular Arbuscular mycorrhiza in rhizomes scale-like leaves, roots and xylem of ginger. Mycologia 74:156–161

Taylor TN, Remy W, Hass H, Kerp H (1993) Fossil Arbuscular mycorrhizae from the early devonian. Mycologia 87:560–573

Thomas E, Jalonen R, Loo J, Boshier D, Gallo L, Cavers S, Bordacs S, Smith P, Bozzano M (2014) Genetic considerations in ecosystem restoration using native tree species. For Ecol Manage 333:66–75

Treseder KK, Turner KM (2007) Glomalin in ecosystems. Soil Sci Soc Am J 71:1257–1266

UN (2015) Transforming our world: the 2030 agenda for sustainable development. Available via https://sustainabledevelopment.un.org/post2015/transformingourworld

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

Veiga RSL, Jansa J, Frossard E, van der Heijden MGA (2011) Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? PLoS One 6:e27825. https://doi.org/10.1371/journal.pone.0027825

Verma NK (1998) Effect of VA mycorrhiza on the growth and P uptake in *Eupatorium adenophorum* Spring. (Asteraceae) grown in soil amended with soluble phosphate. J Natl Bot Soc 52:41–45

Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363. https://doi.org/10.1007/s00572-005-0033-6

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198:97–107

Wu JG, Loucks O (1992) In: U.S. National Research Council (ed) Grasslands and grassland sciences in Northern China. National Academy Press, Washington, DC

Wubet T, Wei BM, Kottke I, Oberwinkler F (2006) Two threatened coexisting indigenous conifer species in the dry afromontane forests of Ethiopia are associated with distinct arbuscular mycorrhizal communities. Can J Bot 84:1617–1627. https://doi.org/10.1139/b06-121

Wubet T, Kottke I, Teketay D, Oberwinkler F (2009) Arbuscular mycorrhizal fungal community structures differ between co-occurring tree species of dry Afromontane tropical forest, and their seedlings exhibit potential to trap isolates suited for reforestation. Mycol Prog 8:317–328. https://doi.org/10.1007/s11557-009-0602-8

Yoshino M (2001) Relationship between land degradation and sand dust storm occurrence, aeolian sand transport and its damages in East Asia during the recent years. In Adeel Z (ed) Integrated land management in dry areas (UNU Desertification Series No. 4). Kinkosha Printers, Tokyo, pp 119–136

Zhou G, Wang Y, Wang S (2002) Response of grassland ecosystems to precipitation and land use along the Northeast China Transect. J Veg Sci 13:361–368

Zhou HR, Zhao XQ, Tang YH, Song G, Li Z (2005) Alpine grassland degradation and its control in the source region of the Yangtze and Yellow River, China. Jpn Soc Grassl Sci 51:191–203

Chapter 23
The Role Played by Mycorrhizal Fungi in Ecorestoration

Bidisha Sharma and Dhruva Kumar Jha

Abstract The pivotal role played by arbuscular mycorrhizal fungi (AMF) in maintaining plant and soil health has made this group of beneficial fungi a star celebrity amongst a host of beneficial microbes used by human brain for its welfare. Now days because of their myriad beneficiary effects especially on the plant health, they are considered as one of the most potent biological tools for restoration purposes. This review hence focused on the role played by AMF on eco restoration, reaction of AMF to their hosts and the myriad challenges faced by different workers for mycorrhizal application in degraded soils.

23.1 Introduction

Mahatma Gandhi once said "earth produces enough for one's need but not enough for one's greed". These words of Gandhiji have been very much reflected through the various anthropogenic activities carried out for urbanization. Urbanization works like making of high ways, establishment of industries and building of high rises are leading to large scale deforestation, extreme pollution of the environment and degradation of various ecosystems. Such destruction caused by human in the name of urbanization is just a reflection of their greed and not need. As the natural ecosystems provide human society not only food, fuel and timber but also purify air and water, detoxify and decompose wastes, regulate climate, regenerate soil fertil ity and pollinate crops, it is, therefore, the utmost responsibility of the human beings to conserve these natural systems as well as restore the ones which have been degraded or destroyed. The level of degradation is often measured in terms of reduction of net primary productivity (Bai et al. 2008) but now the Society for Ecological Restoration (SER 2004) has recommended attributes such as low biomass, low soil organic matter, poor soil water relationship etc. for measurement of degradation.

B. Sharma • D.K. Jha (✉)
Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati 781014, Assam, India
e-mail: dkjhabot07@gmail.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_23

Conservation practices often help in mitigating the negative effects of human activities. Hence, efforts are being made to conserve the degraded ecosystems applying different conservation strategies, especially using green tools like microbes, plant growth regulators, etc. In the 1980s, interest grew for restoration of ecosystems and it resulted in development of ecological field called restoration ecology. It is the scientific study supporting the practice of ecological restoration through renewal and restoration of degraded, damaged, or destroyed ecosystems and habitats in the environment by active human intervention and action. Ecological restoration can be considered as a means of sustaining the diversity of life on Earth and re-establishing an ecologically healthy relationship between nature and culture. Hence, ecological restoration should be a taken as a fundamental component of conservation and sustainable development programs throughout the world by virtue of its inherent capacity to provide people with the opportunity to not only repair ecological damage, but also improve the human condition.

Ecological restoration has now emerged as one of the central themes of global environmental policies (Aradottir and Hagen 2013; Jacobs et al. 2015). Restoration of at least 15% of the world's degraded ecosystems is one of the 20 targets of the UN Convention on Biological Diversity (CBD 2010). Restoration experiences so far show that many restoration projects achieve limited success or fail completely (Thomas et al. 2014). Such failure can be attributed to absence of green tools like beneficial microorganisms. The beneficial microbes help in the restoration processes as they are responsible for making soil nutrients available to plants, fix nitrogen, increase phosphorous uptake of the plant, degrade residual pesticides and so on. Therefore, efforts involving use of beneficial microbes are needed for achieving successful restoration.

The degraded soils often lead to various stresses like water stress, nutrient deficiency, acidic root environment etc. to the plant present in that soil. Hence, beneficial plant associated microbes such as arbuscular mycorrhizal fungi (AMF), PGPRs etc. for restoration purposes and *in situ* management for better restoration outcome of degraded lands. AMF having ubiquitous presence in the rhizosphere of about 93% of flowering plant families (Wang and Qiu 2006; Brundrett 2009) act as keystone mutualists with myriad roles in an ecosystem. The external hyphae network (extra radical mycelium) of the fungi permeates into the microsites of rocks and soils surrounding the plant roots (Finlay 2008; Barea et al. 2011) increasing the root absorbing surface area 100 or even 1000 fold (Larcher 1995). Therefore, AMF increase plants nutrient and water relation (Birhane et al. 2012, 2015; Banerjee et al. 2013), and can improve plants' field survival and establishment (Pouyu-Rojas and Siqueira 2000; Habte et al. 2001; Ouahmane et al. 2006; Dag et al. 2009; Kapulnik et al. 2010; Karthikeyan and Krishnakumar 2012; Manaut et al. 2015). AMF improve soil structure, soil water relation, plants' tolerance to biotic and abiotic stresses, increase plants' nutrient supply, plants' growth, yield and reproductive success and reduce fertilizer requirement (Finlay 2008; Gianinazzi et al. 2010; Simard and Austin 2010; Barea et al. 2011; Al-Karaki 2013; Soka and Ritchie 2014). AMF influence plant community structure (van Der Heijden et al. 1998; Hartnett and Wilson 1999; Renker et al. 2004; Heneghan et al.

2008; Lin et al. 2015) and are considered to have a pivotal role in plant community assembly and succession (Janos 1980; Renker et al. 2004; Kikvidze et al. 2010).

The beneficial effects of AM fungi on plant performance and soil health can be harnessed for human welfare by applying them in sustainable management of agricultural ecosystems (Jeffries et al. 2003; Barrios 2007), ecosystem restoration (van der Heijden et al. 1998), bioaugmentation of plants of economic importance (Amaranthus et al. 2009) and so on. Hence, AMF inoculation has grown to be a biotechnological tool that is widely applicable in ecological restoration.

The myriad roles played by AMF in improvement of soil health and plant performance have encouraged restoration technologists to use them as a green tool. As such this review has been written to focus on how AMF interaction with the plants help in eco-restoration purposes and what are the various challenges faced by biotechnologists for AMF application.

23.2 Mechanism of Plant–AMF Interaction

The AMF interaction with plants can be categorized as a symbiotic or mutualistic interaction. The rhizosphere of the mycorrhiza infected plants is known as mycorrhizosphere (Barea et al. 2002) and it provides a critical link between plants, other microorganisms and the soil (Hrynkiewicz and Baum 2011). Intricate interactions like interactions between AMF and the plant, AMF and bacteria, AMF and other fungi, and among AMF themselves take place within the mycorrhizosphere.

These interactions occurs when plant roots exude trigolactones (SLs) (Parniske 2008; Gutjahr 2014) which induce AMF spore germination and hyphal branching (Parniske 2008). The AMF on their part, produce mycorrhiza (*Myc*) factors which induce calcium oscillations in root epidermal cells and also activate plant symbiosis-related genes (Parniske 2008). Then the AMF form special type of appressoria called hyphopodia which develops from mature hyphae (Parniske 2008) and guides the fungus through root cells toward the cortex. The fungus then leaves the plant cell and enters the apoplast, where it branches and grows laterally along the root axis (Parniske 2008; Gutjahr 2014) and forms arbuscles and vesicles.

Arbuscular mycorrhizal fungi are known to play role in plant nutrition as long as they collaborate with other soil microbes (Barea et al. 2002; Antunes et al. 2007). Arbuscular mycorrhizal fungi also interact with decomposer fungi (Soka and Ritchie 2014) and phosphate solubilizing fungi (PSF) synergistically (Osoria and Habte 2001). AMF are also known to have antagonistic relationship with root pathogens (Soka and Ritchie 2014) and even leaf pathogens (Parniske 2008). Arbuscular mycorrhizal fungi may also interact with each other synergistically. It was experimentally found out that AMF effects are greater when AMF consortia inoculums are applied than single AMF (Barea et al. 2011; Banerjee et al. 2013, Sharma and Jha 2015). Improved access to scarce soil resources, especially

phosphorus, is often the main mechanism through which AMF benefits their host plants (Smith and Read 1997; Schultz et al. 2001; Miller et al. 2002; Johnson et al. 2010).

23.3 AMF and Eco-restoration

The pivotal role played by AMF in eco-restoration involves improved plant fitness, improved nutrient uptake and accumulation, increased tolerance of adverse conditions and altering plant community structure (Brundrett and Abbott 2002). However, the use of AMF, especially the native ones, for ecosystem restoration has proved to be more successful than use of exotic ones (Requena et al. 2001; Vogelsang et al. 2006) as native fungi have the capacity to promote growth as well as improve water use efficiency (Querejeta et al. 2006) and also impart tolerance to herbivory (Bennett and Bever 2007; Bennett et al. 2009).

AMF contributes towards ecorestoration by being one of the most effective soil organisms in stabilizing soil structure (Augé 2004; Al-Karaki 2013) through its hyphae. AMF also improve soil aggregation by influencing bacterial communities that can improve soil aggregate formation (Rilling 2004). Furthermore, the dead AMF hyphae produce glomalin which is hydrophobic stable aggregate (Barea et al. 2002; Simard and Austin 2010). Hence, AMF increase both soil aggregation and stability. Further, AMF increase soil organic matter content and stability (Leifheit et al. 2014). Improved soil aggregation also increases soil water relation (Marschner 1995).

Another attribute of AMF which makes them beneficial ecorestoration tool is their role in phosphorous and nitrogen nutrition (Skujins and Allen 1986; Requena et al. 2001; Govindarajulu et al. 2005). AMF were observed to improve potassium nutrition too in plants (Dag et al. 2009; Garcia and Zimmermann 2014). AMF can also increase the uptake of other macro and micro nutrients by plants (Birhane et al. 2012) by establishment of an underground network that links the different plants and hence sequester carbon, nitrogen and phosphorous and also allow the transfer of these nutrients among plants (Rodriguez-Echeverria et al. 2007). AMF also reduce nutrient leaching from the soil (Rodriguez-Echeverria et al. 2007).

Another benefit of AMF as eco-tool is that they can increase plants' tolerance to drought and salinity (Al-Karaki 2013). As for e.g. by inoculating plants with drought tolerant AMF, up to 42% reduction in plants' water requirement could be achieved (Gianinazzi et al. 2010). AMF are also known to increase heavy metal stress in plants (Leyval et al. 1997; Hildebrandt et al. 2007; Soares and Siqueira 2008; Amir et al. 2013). The mechanism by which AMF increase plants' tolerance to drought, salinity and heavy metal stresses is mainly nutritional (Marschner 1995; Soares and Siqueira 2008; Birhane et al. 2012; Al-Karaki 2013; Navarro et al. 2013). The non-nutritional mechanisms by which AMF increase plants' tolerance to drought include hormonal changes, hyphal soil improvement (delayed soil drying), hyphal ability to scavenge water from micro-pores, increased plants'

photosynthetic rate etc. (Marschner 1995; Birhane et al. 2012; Al-Karaki 2013). Likewise, immobilizing heavy metals in their biomass mainly cell wall, vesicles and in the glomalin is the non-nutritional mechanism by which AMF improve plants' tolerance to heavy metals stress (Hildebrandt et al. 2007). The positive AMF effects on plants' drought tolerance can improve plants' salinity tolerance as well. Other non-nutritional mechanisms by which AMF improve plants' salinity tolerance include exclusion of salt from plant cells by accumulating the salt within the fungal hyphae, production of enzymes involved in antioxidant defense, and change in cell wall elasticity and membrane stability (Al-Karaki 2013).

Another important attribute of AMF contributing towards the eco-restoration is their ability to make plants stress resistant. Studies across more than 144 published papers supports this view. As the role AMF is pivotal bioprotection, hence, Gianinazzi et al. (2010) described AMF as 'health insurance' of plants. One mechanism by which AMF increase plants' pathogen tolerance could be the synergistic interaction of AMF have with plant growth promoting rhizobacteria (PGPR). Further, AMF stimulate the synthesis of plant secondary metabolites (Gianinazzi et al. 2010) which aid in inhibition of herbivory. The other reason by which AMF increase plants' herbivory tolerance is compensatory growth.

An analysis of different published papers (Pouyu-Rojas and Siqueira 2000; Habte et al. 2001; Lekberg and Koide 2005; Ouahmane et al. 2006; Dag et al. 2009; Kapulnik et al. 2010; Karthikeyan and Krishnakumar 2012; Manaut et al. 2015) on contribution of AMF towards plant growth and productivity revealed that AMF generally increase growth and productivity of plants. As AMF can significantly improve tree seedlings field survival and establishment hence AMF proves to be an important tool for restoration of degraded lands.

The mycorrhizal community also influences the succession of plants in a particular site (Janos 1980; Zobel and Öpik 2014). However, it has been observed that the plant community also influences the AMF community of that particular site (Renker et al. 2004). Thus, there is always need of specific compatible relationships between AMF and plant taxa and the loss of compatible AMF species or individuals may limit the distribution of a particular plant species (Renker et al. 2004). AMF further influence plant community structure by affecting richness or evenness of co-existing plants (Brundrett and Abbott 2002). AMF have no host specificity as only about 240 AMF morpho-species have been described forming associations with 80% of terrestrial plants (Lee et al. 2013) and a single fungus can link different plants together, thereby forming mycorrhizal networks (Simard and Austin 2010; Song et al. 2014). These networks help in regeneration of new seedlings, alter species interactions, and change the dynamics of plant communities therefore, increasing plant diversity (Simard and Austin 2010). AMF may also aid in inhibiting invasion by alien species.

23.4 Use of AMF for the Restoration of Degraded Lands

The degraded lands usually have low level of AMF and therefore, plants with AMF inoculation could potentially be considered as an important biotechnological tool in degraded land restoration. As AMF show non-host specificity to forge symbiotic relationship with plants and are ubiquitous (Abbott and Robson 1991; Brundrett and Abbott 2002; Barea et al. 2011; Al-Karaki 2013), hence, many researchers argue that AMF inoculation is likely to be valuable in only few conditions such as mine fields where indigenous AMF inoculum is surely little or none available (Brundrett and Abbott 2002). As such Koide and Mosse (2004) emphasized on managing the indigenous AMF population of a site which can be quite economical and appropriate to focus on. AMF inoculation has proved to be effective under wide range of soil conditions (Janos 1980; Brundrett and Abbott 2002; Banerjee et al. 2013). Veiga et al. (2011) demonstrated that AMF inoculation suppressed weeds and hypothesized that AMF inoculation could suppress wild plants which can invade degraded sites and compete with planted seedlings.

Though the use of native AMF inocula is preferred over the use of exotic inocula but the several stage of the native AMF should be considered as early seral AMF spores are better for restoration purposes (Allen et al. 2003). The early seral AMF having small spores have smaller carbon demand (Allen et al. 2003) as compared to the late seral AMF which have big spores and demand much carbon and hence, seedlings may not benefit from them.

Though AMF is a powerful tool for eco-restoration purpose but the challenge associated with the use of AMF biotechnology is related with inocula production for large-scale application. As AMF is obligate in nature hence, they cannot be cultured axenically (Azcón-Aguilar et al. 1999; Fortin et al. 2005) and host plant based AMF multiplication is mandatory. Further, these host plant based inocula production methods are costly and large scale production of AMF inocula may hardly be possible. However, in India, monexenic *in vitro* culturing of AMF has been used for large-scale industrial production of biologically clean AMF inocula (Adholeya et al. 2005).

23.5 Ecorestoration with Mycorrhizal Application

Over the last few years, AMF is increasingly used for eco-restoration or environment improvement (van der Heijden et al. 1998; Streitwolf Engel et al. 2001; Hajboland et al. 2010). Such improvement is because of different attributes of the AMF such as when mycelium is formed between AMF and its host plant, the soil contact area of host plant roots is expanded, resulting in improved nutrient and water absorption of plant, and increased stress tolerance (Fokoma et al. 2012; Spohn and Giani 2010; Augé 2001). Further, mycorrhizal inoculation also significantly increases the content of microorganisms in the rhizospheric soil, thereby

improving the rhizospheric micro environment of the host plants and resulting into improvement of soil productivity and ecological reconstruction (Lin et al. 2015).

Various human activities like construction, agriculture, and management practices alter AMF density and community composition thereby, promoting exotic plant invasion (Cousins et al. 2003; Kulmatiski et al. 2006; Vogelsang and Bever 2009). Degradation of AMF communities also leads to slower success of native plant community restorations, as native plants often have high dependence on AMF (Wilson and Hartnett 1998; Vogelsang et al. 2006; Vogelsang and Bever 2009; Koziol and Bever 2015) and therefore inoculation with AMF may increase the diversity of native plants within restorations (Clemente et al. 2004). Several pot studies have suggested that native plants perform better with AMF communities derived from the native plants' habitat (Fitzsimons and Miller 2010; Mangan et al. 2010; Taheri and Bever 2011; Johnson et al. 2012). The degree to which a plant is colonized by commercially produced inoculum also depends on the commercial product, growth medium, and greenhouse conditions (Corkidi et al. 2004). Some studies evaluating plant response to local AMF in the field have shown a positive response to AMF derived from native remnant communities in the restoration of native vegetation (Williams et al. 2011; Zhang et al. 2012; Estrada et al. 2013).

There have been many successful stories involving AMF inoculation for restoration purposes as are discussed below. Bever et al. (2003) observed that inoculation with inoculum derived from native Prairie soil increased the plant diversity more than inoculum from old fields or sterilized soil, suggesting that native locally adapted inocula could be important. Several studies found the response of native plants to commercially produced inoculum poor as compared to whole soil (containing native AMF) in the field (Rowe et al. 2007; Paluch et al. 2013). Further, understanding the variation in response to AMF inoculation across plant successional status may have important restoration implications. Several studies suggested that early successional plants tend to be less responsive to mycorrhizal colonization as compared to late successional plants because investment in a mutualism has short-term costs but long-term benefits (Janos 1980; Allen and Allen 1984, 1988; Hoeksema et al. 2010). Work of Middleton and Bever (2012) found that the response to inoculation with native whole soil depended on the succession status of the native Prairie plant species, with early successional plants responding negatively and mid-and late-successional plants responding positively.

Though numerous studies have examined the effects of disturbance on soil microflora and fauna (e.g., Wardle et al. 1995; Brussaard et al. 1997), and soil biota are commonly employed as indicators of restoration success (Andersen and Sparling 1997; Todd et al. 2006; Callaham et al. 2008) yet, soil fauna have rarely been directly manipulated to improve restoration success. However, the use of mycorrhizal fungi in restoration has attracted considerable attention in recent years, and increasingly sophisticated knowledge of this group of soil organisms can influence restoration. It has been found that the community and ecosystem consequences of mycorrhizal infection vary with mycorrhizal dependency of the dominant and rare species in a community (Bever et al. 2001; Bever 2002). For

example, if dominant species depend on mycorrhizae, then their presence may be necessary for restoring ecosystem function (Richter and Stutz 2002). Restoration of rare species that are mycorrhizae dependent may require inoculation for establishment and achieving the desired community composition (van der Heijden et al. 1998). Likewise, inoculation may be necessary to reclaim extremely degraded sites and maximize productivity of a limited species pool under such circumstances (Frost et al. 2001). Use of mycorrhizae in restoration requires an understanding of ecological consequences from the relationship between below ground organisms, above ground individuals, community structure, and ecosystem processes.

23.6 Challenges for Mycorrhizal Application

Though restoration of degraded soils using mycorrhizae is difficult (Cardoso and Kuyper 2006) yet there is growing interest in using commercial mycorrhizae inoculums to improve restoration success due to their myriad beneficial attributes. However, application of mycorrhizae requires knowledge about site conditions as they may or may not grow in a particular site. As for e.g. AMF may not grow at sites contaminated with heavy metals or where nutrients are very low (Vosátka et al. 1999). Additionally, they may also be inhibited by high levels of nutrients such as nitrogen from vehicles and fertilizers (Egerton-Warburton et al. 2007).

For effective incorporation of mycorrhizae as a restoration tool, a moderate level of knowledge about the interactions between the physical, chemical, and biological factors prevailing at a site is needed. A major concern with respect to inoculation of commercially produced, exotic AMF is that they will suppress native AMF and potentially become invasive (Schwartz et al. 2006; Pringle et al. 2009). Study by Middleton et al. 2015 showed that inoculation with non-locally adapted, commercial strains of AMF suppressed the colonization of the plants by resident AMF. The duration for the spread of commercial AMF within an ongoing restoration site should be monitored so as to prevent them from becoming invasive problems.

Some view ecosystem restoration as impractical, in part because it sometimes fails. According to Hilderbrand et al. (2005) many times uncertainty about the ecosystem functions is not addressed, and that the time-scales set out for ‘complete’ restoration is unreasonably short. In some other cases, an ecosystem may be so degraded that abandonment i.e. allowing an injured ecosystem to recover on its own may be the best option (Holl 2006). High economic costs can also be perceived as a negative impact of the restoration process (MacDonald et al. 2002).

23.7 Conclusion

As discussed above, AMF is contributing immensely towards the maintenance of plant community in an ecosystem and as such it is proving to be a strong biological tool for eco-restoration. As there have been success stories of implementing AMF in restoration of degraded soils hence, efforts should be put forth for more such use of AMF for restoration purposes. However, the successful implementation of this biological tool depends entirely on the strategies of restoration ecologists.

Restoration ecologists not only provide appropriate conditions for desired species to establish but they also devise ways of preventing the establishment by invasive plants or weeds (Zedler 2005). Further, it has been observed that some low level of natural disturbance (e.g. logging, fire, flooding, etc.) can enhance biological diversity and hence, encourage ecological restoration (Palmer et al. 1997).

Ecological restoration ranges from species reintroduction to population restoration to community restoration (Young et al. 2001). Based on the restoration goal, it ranges from reclamation to rehabilitation to true restoration. As the magnitude of disturbance increases the return to pre-disturbance status may be impossible and hence, return to an intermediate successional status of the given community may be achieved i.e. rehabilitation may occur. When the disturbance is severe, the threshold of irreversibility is passed and the return to pre-disturbance community status or intermediate successional status will be completely impossible and hence, restoration can only result in a novel community stature (reclamation) (Aronson et al. 1993). In the advent of climate change, to have reclamation as a restoration goal is considered to be relevant since novel climatic conditions are anticipated in the future (Choi 2004). In tropical lands ecological restoration, tree planting (Lamb et al. 2005; Holl et al. 2010; Aerts and Honnay 2011) and re-vegetation/reforestation (Cortines and Valcarcel 2009; Al-Karaki 2013) are known to be the most effective and widely used biological measures.

Further, observing the myriad roles played by AMF in plant life, it is possible to conclude that AMF inoculation can significantly increase the success of degraded land restoration.

23.8 Future Prospect

The role of Restoration ecology is botanically based (Young 2000) and results in greater emphasis on the role of soil's physical and microbial processes (Allen et al. 2003). Human intervention in this case is used to promote habitat, biodiversity recovery and associated gains. Human effort with the use of AMF will lead to better results. AMF biotechnology is a potential mechanism to significantly improve the restoration procedure of a degraded land. As AMF restores degraded lands by virtue of improvement in plant and soil health, hence future research efforts should focus

on various aspects of AMF–plant interaction as well as production of cost-effective inocula which can be applied for the restoration of degraded ecosystems. Emphasis should also be given on shortening the duration of restoration process.

References

Abbott LK, Robson AD (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric Ecosyst Environ 35:121–150

Adholeya A, Tiwari P, Singh R (2005) Large scale inoculum production of arbuscular mycorrhizal fungi on root organs and inoculation strategies. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 315–338

Aerts R, Honnay O (2011) Forest restoration, biodiversity and ecosystem functioning. BMC Ecol 11:29

Al-Karaki GN (2013) The role of mycorrhiza in the reclamation of degraded lands in arid environments. In: Shahid SA, Taha FK, Abdelfattah MA (eds) Developments in soil classification, land use planning and policy implications: innovative thinking of soil inventory for land use planning and management of land resources. Springer Science C Business Media, Dordrecht, pp 823–836

Allen EB, Allen MF (1984) Competition between plants of different successional stages: mycorrhizae as regulators. Can J Bot 62:2625–2629

Allen EB, Allen MF (1988) Facilitation of succession by the nonmycotrophic colonizer Salsola kali (Chenopodiaceae) on a harsh site: effects of mycorrhizal fungi. Am J Bot 75:257–266

Allen EB, Allen MF, Egerton-Warburton L, Corkidi L, Gomez-Pompa A (2003) Impacts of early and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. Ecol Appl 13:1701–1717

Amaranthus MP, Simpson L, Landis TD (2009) How mycorrhizae can improve plant quality. Comb Proc Int Plant Propagators Soc 34:33–38

Amir H, Lagrange A, Hassaïne N, Cavaloc Y (2013) Arbuscular mycorrhizal fungi from New Caledonian ultra-mafic soils improve tolerance to nickel of endemic plant species. Mycorrhiza 23:585–595

Andersen AN, Sparling GP (1997) Ants as indicators of restoration success: relationship with soil microbial biomass in the Australian seasonal tropics. Restor Ecol 5:109–114

Antunes PM, Schneider K, Hillis D, Klironomos JN (2007) Can the arbuscular mycorrhizal fungus *Glomus intraradices* actively mobilize P from rock phosphates? Pedobiologia 51:281–286

Aradottir AL, Hagen D (2013) Ecological restoration: approaches and impacts on vegetation, soils and society. Adv Agron 120:173–222

Aronson J, Fled C, LeFloc'h E, Ode C, Pontanier R (1993) Restoration and rehabilitation of degraded ecosystems in arid and semi-arid lands: a view from the south. Restor Ecol 1:8–17

Augé RM (2001) Water relations, drought and VA mycorrhizal symbiosis. Mycorrhiza 11:3–42

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Azcón-Aguilar C, Bago B, Barea JM (1999) Saprophytic growth of arbuscular mycorrhizal fungi. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, New York, pp 391–407

Bai ZG, Dent DL, Olsson L, Schaepman ME (2008) Proxy global assessment of land degradation. Soil Use Manag 24:223–234

Banerjee K, Gadani MH, Srivastava KK, Verma N, Jasrai YT, Jain NK (2013) Screening of efficient arbuscular mycorrhizal fungi for *Azadirachta indica* under nursery condition: a step towards afforestation of semi-arid region of Western India. Braz J Microbiol 44:587–593

Barea JM, Azcon R, Azcón-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81:343–351
Barea JM, Palenzuela J, Cornejo P, Sánchez-Castro I, Navarro-Fernández C, Lopéz-Garcia A, Estrada B, Azcón R, Ferrol N, Azcón-Aguilar C (2011) Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. J Arid Environ 75:1292–1301
Barrios E (2007) Soil biota, ecosystem services and land productivity. Ecol Econ 64:269–285
Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218
Bennett AE, Bever JD, Bowers MD (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia 160:771–779
Bever JD (2002) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244:281–290
Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. Bioscience 51:923–931
Bever JD, Schultz PA, Miller RM, Gades L, Jastrow JD (2003) Prairie mycorrhizal fungi inoculant may increase native plant diversity on restored sites (Illinois). Ecol Restor 21:311–312
Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904
Birhane E, Kuyper TW, Sterck FJ, Gebrehiwot K, Bongers F (2015) Arbuscular mycorrhiza and water and nutrient supply differently impact seedling performance of acquisitive and conservative dry woodland species. Plant Ecol Divers 8:1–13
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
Brundrett MC, Abbott LK (2002) Arbuscular mycorrhiza in plant communities. In: Sivasithamparam K, Dixon KW, Barrett RL (eds) Plant conservation and biodiversity. Kluwer Academic Publishers, Dordrecht, pp 151–193
Brussaard L, Behan-Pelletier VM, Bignell DE, Brown VK, Didden W, Folgarait P (1997) Biodiversity and ecosystem functioning in soil. Ambio 26:563–570
Callaham M, Rhoades CR, Heneghan L (2008) A striking profile: soils knowledge in restoration management and science. Restor Ecol 16:604–607
Cardoso IM, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agric Ecosyst Environ 116:72–84
CBD (2010) Aichi biodiversity targets. Available via http://www.cbd.int/sp/targets/
Choi YD (2004) Theories for ecological restoration in changing environment: toward 'futuristic' restoration. Ecol Res 19:75–81
Clemente AS, Werner C, Maguas C, Cabral MS, Martins-Loucao MA, Correia O (2004) Restoration of a limestone quarry: effect of soil amendments on the establishment of native Mediterranean sclerophyllous shrubs. Restor Ecol 12:20–28
Corkidi L, Allen EB, Merhaut D, Allen MF, Downer J, Bohn J, Evans M (2004) Assessing the infectivity of commercial mycorrhizal inoculants in plant nursery conditions. J Environ Hortic 22:149–154
Cortines E, Valcarcel R (2009) Influence of pioneer-species combinations on restoration of disturbed ecosystems in the Atlantic Forest, Rio de Janeiro, Brazil. Rev Arvore 33:927–936
Cousins JR, Hope D, Gries C, Stutz JC (2003) Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem. Mycorrhiza 13:319–326
Dag A, Yermiyahu U, Ben-Gal A, Zipori I, Kapulnik Y (2009) Nursery and post-transplant field response of olive trees to arbuscular mycorrhizal fungi in an arid region. Crop Pasture Sci 60:427–433
Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecol Monogr 77:527–544

Estrada B, Aroca R, Azcon-Aguilar C, Barea JM, Ruiz-Lozano JM (2013) Importance of native arbuscular mycorrhizal inoculation in the halophyte, *Asteriscus maritimus* for successful establishment and growth under saline conditions. Plant Soil 370:175–185

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J Exp Bot 59:1115–1126

Fitzsimons MS, Miller RM (2010) The importance of soil microorganisms for maintaining diverse plant communities in tallgrass prairie. Am J Bot 97:1937–1943

Fokoma R, Adamou S, Teugwa MC, Begoude Boyogueno AD, Nana WL, Ngonkeu MEL, Tchameni NS, Nwaga D, Tsala Ndzomo G, Amvam Zollo PH (2012) Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land use variation in the humid forest zone of south Cameroon. Soil Tillage Res 120:69–75

Fortin JA, Declerck S, Strullu DG (2005) In vitro culture of mycorrhizas. In: Declerck S, Strullu DG, Fortin A (eds) In vitro culture of mycorrhizae. Springer, Berlin, pp 3–14

Frost SM, Stahl PD, Williams SE (2001) Long-term reestablishment of arbuscular mycorrhizal fungi in a drastically disturbed semiarid surface mine soil. Arid Land Res Manag 15:3–12

Garcia K, Zimmermann SD (2014) The role of mycorrhizal associations in plant potassium nutrition. Front Plant Sci 5:337. https://doi.org/10.3389/fpls.2014.00337

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

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nat Lett 435:819–823

Gutjahr C (2014) Phytohormone signaling in arbuscular mycorrhiza development. Curr Opin Plant Biol 20:26–34

Habte M, Miyasaka SC, Matsuyama DT (2001) Arbuscular mycorrhiza fungi improve early forest-tree establishment. In: Horst WJ, Schenk MK, Bürkert A, Claassen N, Flessa H, Frommer WB, Goldbach H, Olfs H-W, Römheld V, Sattelmacher B, Schmidhalter U, Schubert, S., von Wirén N, Wittenmayer L (eds) Plant nutrition: food security and sustainability of agroecosystems through basic and applied research. Springer

Hajboland R, Aliasgharzadeh N, Laiegh SF, Charlotte P (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. Plant Soil 331:313–327

Hartnett DC, Wilson GWT (1999) Mycorrhiza influence plant community structure and diversity in tall grass Prairie. Ecology 80:1187–1195

Heneghan L, Miller SP, Baer S, Callaham MA, Montgomery J, Pavao-Zuckerman M (2008) Integrating soil ecological knowledge into restoration management. Restor Ecol 16:608–617

Hildebrandt U, Regvar M, Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146

Hilderbrand RH, Watts AC, Randle AM (2005) The myths of restoration ecology. Ecol Soc 10:19

Hoeksema JD, Chaudhary VB, Gehring CA et al (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407

Holl K (2006) Professor of environmental studies at the University of California. Santa Cruz, Personal Communication

Holl KD, Zahawi RA, Cole RJ, Ostertag R, Cordell S (2010) Planting seedlings in tree islands versus plantations as a large-scale tropical forest restoration strategy. Restor Ecol 19:470–479

Hrynkiewicz K, Baum C (2011) The potential of rhizosphere microorganisms to promote the plant growth in disturbed soils. In: Malik A, Grohmann E (eds) Environmental protection strategies for sustainable development, strategies for sustainability. Springer Science C Business, New York, pp 35–64

Jacobs DF, Oliet JA, Aronson J, Bolte A, Bullock JM, Donoso PJ (2015) Restoring forests: what constitutes success in the twenty-first century? New For 46:601–614

Janos DP (1980) Mycorrhizae influence tropical succession. Biotropica 12:56–64

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

Johnson NC, Wilson GWT, Bowker MA, Wilson T, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci USA 107:2093–2098

Johnson DJ, Beaulieu WT, Bever JD, Clay K (2012) Conspecific negative density dependence and forest diversity. Science 336:904–907

Kapulnik Y, Tsror L, Zipori I, Hazanovsky M, Wininger S, Dag A (2010) Effect of AMF application on growth, productivity and susceptibility to *Verticillium* wilt of olives grown under desert conditions. Symbiosis 52:103–111

Karthikeyan A, Krishnakumar N (2012) Reforestation of bauxite mine spoils with *Eucalyptus tereticornis* Sm. seedlings inoculated with arbuscular mycorrhizal fungi. Ann For Res 55:207–216

Kikvidze Z, Armas C, Fukuda K, Martínez-García LB, Miyata M, Oda-Tanaka A (2010) The role of arbuscular mycorrhizae in primary succession: differences and similarities across habitats. Web Ecol 10:50–57

Koide RT, Mosse B (2004) A history of research on arbuscular mycorrhiza. Mycorrhiza 14:145–163

Koziol L, Bever JD (2015) Mycorrhizal response trades off with plant growth rate and increases with plant successional status. Ecology 96:1768–1774

Kulmatiski A, Beard KH, Stark JM (2006) Soil history as a primary control on plant invasion in abandoned agricultural fields. J Appl Ecol 43:868–876

Lamb D, Erskine PD, Parrotta JD (2005) Restoration of degraded tropical forest landscapes. Science 310:1628–1632

Larcher W (1995) Physiological plant ecology. Springer, Berlin

Lee E-H, Eo J-K, Ka K-H, Eom A-H (2013) Diversity of arbuscular mycorrhizal fungi and their roles in ecosystems. Mycobiology 41:121–125

Leifheit EF, Verbruggen E, Rillig MC (2014) Arbuscular mycorrhizal fungi reduce decomposition of woody plant litter while increasing soil aggregation. Soil Biol Biochem 81:323–328

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytol 168:189–204

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Lin G, Mc Cormack ML, Guo D (2015) Arbuscular mycorrhizal fungal effects on plant competition and community structure. J Ecol 103:1224–1232

MacDonald DW, Moorhouse TP, Enck JW (2002) The ecological context: a species population perspective. Cambridge University Press, Cambridge

Manaut N, Sanguin H, Ouahmane L, Bressan M, Thioulouse J, Baudoin E (2015) Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. Ecol Eng 79:113–119

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant-soil feedback predicts tree species relative abundance in a tropical forest. Nature 466:752–755

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

Middleton EL, Bever JD (2012) Inoculation with a native soil community advances succession in a grassland restoration. Restor Ecol 20:218–226

Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, Henning JA, Schultz PA, Bever JD (2015) Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. Ecosphere 6:1–16

Miller RM, Miller SP, Jastrow JD, Rivetta CV (2002) Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. New Phytol 155:149–162

Navarro JM, Perez-Tornero O, Morte A (2013) Alleviation of salt stress in Citrus seedlings inoculated with arbuscular mycorrhizal fungi depends on the root stock salt tolerance. J Plant Physiol 171:76–85

Osoria NW, Habte M (2001) Synergetic influence of an arbuscular mycorrhizal fungus and P solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in anoxisol. Arid Land Res Manag 1115:263–274

Ouahmane L, Hafidi M, Thioulouse J, Ducousso M, Kisa M, Prin Y (2006) Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. J Appl Microbiol 103:683–690

Palmer MA, Ambrose RF, Poff NL (1997) Ecological theory and community restoration ecology. Restor Ecol 5:291–300

Paluch EC, Thomsen MA, Volk TJ (2013) Effects of resident soil fungi and land use history outweigh those of commercial mycorrhizal inocula: testing a restoration strategy in unsterilized soil. Restor Ecol 21:380–389

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Microbiology 6:763–775

Pouyu-Rojas E, Siqueira JO (2000) Arbuscular mycorrhizal and soil fertilization on post-transplant development of out plants of seven forest species. Pesq Agrop Bras 35:103–114

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal symbioses and plant invasions. Annu Rev Ecol Syst 40:699–715

Querejeta JI, Allen MF, Caravaca F, Roldan A (2006) Differential modulation of host plant delta C-13 and delta O-18 by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. New Phytol 169:379–387

Renker C, Zobel M, Öpik M, Allen MF, Allen EB, Vosátka M (2004) Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? In: Temperton VM, Hobbs RJ, Nuttle T, Halle S (eds) Assembly rules and restoration ecology: bridging the gap between theory and practice. Island Press, Washington, DC, pp 189–229

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

Richter BS, Stutz JC (2002) Mycorrhizal inoculation of Big Sacaton: implications for grassland restoration of abandoned agricultural fields. Restor Ecol 10:607–616

Rilling MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol Lett 7:740–754

Rodriguez-Echeverria S, Costa SR, Freitas H (2007) Biodiversity and interactions in the rhizosphere: effects on ecosystem functioning. In: Pugnaire FI, Valladares F (eds) Functional plant ecology, 2nd edn. CRC Press, pp 581–600

Rowe HI, Brown CS, Claassen VP (2007) Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restor Ecol 15:44–52

Schultz PA, Miller RM, Rivetta C, Jastrow JD, Bever JD (2001) Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* to high and low-nutrient prairies. Am J Bot 88:1650–1656

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

SER (2004) The SER primer on ecological restoration, version 2. Society for Ecological Restoration Science and Policy Working Group. http://www.ser.org/reading_resources.asp

Sharma B, Jha DK (2015) The influence of arbuscular mycorrhizal fungi on the growth of *Psidium guajava* and *Pongamia pinnata*. J Mycol Plant Pathol 45:254–264

Simard SW, Austin ME (2010) The role of mycorrhizas in forest soil stability with climate change. In: Simard SW (ed) Climate change and variability. In Tech Open Access, Rijeka, pp 275–302

Skujins J, Allen MF (1986) Use of mycorrhiza for land rehabilitation. MIRCEN J Microbiol Biotechnol 2:161–176
Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London
Soares CRFS, Siqueira JO (2008) Mycorrhiza and phosphate protection of tropical grass species against heavy metal toxicity in multi-contaminated soil. Biol Fertil Soils 44:833–841
Soka G, Ritchie M (2014) Arbuscular mycorrhizal symbiosis and ecosystem processes: prospects for future research in tropical soils. Open J Ecol 4:11–22
Song YY, Ye M, Li C, He X, Zhu-Salzman K, Wang RL (2014) Hijacking common mycorrhizal networks for herbivore-induced defence signal transfer between tomato plants. Sci Rep 4:1–8
Spohn M, Giani L (2010) Water stable aggregates, glomalin related soil protein, and carbohydrates in a chronosequence of sandy hydromorphic soils. Soil Biol Biochem 42:1505–1511
Streitwolf Engel R, Heijden MGA, Wiemken A, Sanders IA (2001) The ecological significance of arbuscular mycorrhizal fungal effects on clonal reproduction in plants. Ecology 8:2846–2859
Taheri WI, Bever JD (2011) Adaptation of liquid *ambar styraciflua* to coal tailings is mediated by arbuscular mycorrhizal fungi. Appl Soil Ecol 48:251–255
Thomas E, Jalonen R, Loo J, Boshier D, Gallo L, Cavers S (2014) Genetic considerations in ecosystem restoration using native tree species. For Ecol Manag 333:66–75
Todd TC, Powers TO, Mullin PG (2006) Sentinel nematodes of land-use change and restoration in tallgrass Prairie. J Nematol 38:20–27
Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72
Veiga RSL, Jansa J, Frossard E, van der Heijden MGA (2011) Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? PLoS One 6:27825
Vogelsang KM, Bever JD (2009) Mycorrhizal densities decline in association with non-native plants and contribute to plant invasion. Ecology 90:399–407
Vogelsang KM, Reynolds HL, Bever JD (2006) Mycorrhizal fungal identity and richness determine the diversity and productivity of a tall grass prairie system. New Phytol 172:554–562
Vosátka M, Rydlovsá J, Malcovsá R (1999) Microbial inoculations of plants for revegetation of disturbed soils in degraded ecosystems. In: Kovsár P (ed) Nature and culture in landscape ecology. Carolinum Press, Prague, pp 303–317
Wang B, Qiu YL (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363
Wardle DA, Yeates GW, Watson RN, Nicholson KS (1995) The detritus food-web and the diversity of soil fauna as indicators of disturbance regimes in agroecosystems. Plant Soil 170:35–43
Williams A, Ridgway HJ, Norton DA (2011) Growth and competitiveness of the New Zealand tree species *Podocarpus cunninghamii* is reduced by ex-agricultural AMF but enhanced by forest AMF. Soil Biol Biochem 43:339–345
Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. Am J Bot 85:1732
Young TP (2000) Restoration ecology and conservation biology. Biol Conserv 92:73–83
Young TP, Chase JM, Huddleston RT (2001) Succession and assembly as conceptual bases in community ecology and ecological restoration. Ecol Restor 19:5–19
Zedler JB (2005) Ecological restoration: guidance from theory. San Francisco Estuar Watershed Sci 3:1–31
Zhang T, Sun Y, Shi Z, Feng G (2012) Arbuscular mycorrhizal fungi can accelerate the restoration of degraded spring grassland in Central Asia. Rangel Ecol Manage 65:426–432
Zobel M, Öpik M (2014) Plant and arbuscular mycorrhizal fungal (AMF) communities—which drives which? J Veg Sci 25:1133–1140

Chapter 24
Transkingdom Signaling Systems Between Plant and Its Associated Beneficial Microbes in Relation to Plant Growth and Development

Ahlan Sabah Ferdous, Mohammad Riazul Islam, and Haseena Khan

Abstract The concept of plants being holobiont, suggesting a co-existence with their microbial symbionts has emerged only recently with increasing evidences, indicating the versatile role of plant associated microbes in the growth and development of a plant. Therefore plant is no longer considered a single entity but rather a metaorganism, a world of diverse interkingdom interactome. This has led researchers to focus on these associations, with a view to using them in agronomic interventions. Most of these microorganisms are either bacteria or fungi, residing in different parts of a plant, forming a mutualistic relationship with their host. They provide essential nutrients for the growth of plants and protect them against pathogens by acting as the plants' very own army. They allow for a plant's development even in the presence of pathogenic organisms, and also exert an effective role in rescuing plants from the detrimental effects of abiotic stressors. These microorganisms are therefore found to mitigate conditions non-conducive for plant growth. Plants in return, provide them with a secure habitat and adequate source of carbon in order for them to thrive. The interkingdom relationship has been found to be very systematic. Cross talk between the hosts and the benevolent guests is very specific, with particular signaling pathways giving advantage to some microorganisms over others, allowing for the creation of a specific niche. These signals that stimulate plant growth are a focus of intense biological research with more and more information being generated and our understanding of the role of a plant microbiome in overall plant health gradually becoming clearer. This chapter has focused on signaling strategies documented till date between microbes which have their own specific forte in and around a plant. An application of this knowledge would allow for the enhancement of crop production worldwide.

A.S. Ferdous • M.R. Islam • H. Khan (✉)
Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh
e-mail: haseena@du.ac.bd

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_24

24.1 Introduction

Cross-species association is a common phenomenon in the survival of a biosphere, whether parasitic, mutualistic or commensal. This is especially apt for microbes, the tiny organisms (bacteria, fungi etc.) with immense capability of building diverse communication across species of other kingdoms. Vibrant roles performed by microorganisms in supporting the earth's bionetworks add perseverance to our query about this interrelationship. Plant–microbe interaction is one of the most comprehended archetypes of such relations. Research findings over the last several years on microorganisms associated with plants have led to a new concept which now considers plants as metaorganisms. A host of diverse microorganisms which could be endophytes, rhizobial species or phyllosphere inhabiting epiphytes are emerging as the second genome of plants which influence a plant mostly positively in its normal life mode and especially in its tussle to overcome both biotic and abiotic stress conditions. This ties the dynamic microbiome to the health of plants. Any change in the core players of this microbiome is expected to affect a plant negatively. This transkingdom communication has understandably turned into an issue of intense study, to learn how microbes play the role of a friend for the benefit of crops which in many cases continue until senescence. Higher plants have evolved an extremely intricate relationship with microbes of different kinds (Smith and Zhou 2014). A wide range of organic compounds produced by plants including sugars, organic acids and vitamins, can be used as nutrients or signals by microbial populations. Conversely, microorganisms release phytohormones, small molecules or volatile compounds, which may act directly or indirectly to activate plant immunity or regulate plant growth and morphogenesis.

These interactions do not always provide overt evidences. Some microbes do practice a pathogenic relation resulting in some forms which manifest deterioration of plant health. Other types of microbes induce plant immune system to fight against pathogens and appear to be beneficial. Some colonization can be visually seen (root nodules of legumes formed by rhizobia). Yet there are other microbes which act covertly, without any signs or symptoms and provide various essential metabolites, signals and physiological conditions that help the plant to survive. Depending on the plant regions they inhabit and interact, plant associated microbes are variously described. Microbes found in the outer parts of a plant are called epiphytes and those residing within tissues of a plant are called endophytes. Both endophytes and epiphytes are distinguished by the fact that they are asymptomatic. Plant associated microbes can also be classified as phyllomicrobiome (those which are found inside and outside of plant parts that are above ground) and rhizomicrobiomes (those associated with rhizosphere, plant roots and its surrounding areas) (Fig. 24.1). Plants have evolved with microbes over millions of years, and probably, mycorrhizal fungi have co-evolved for at least 400 million years (MY) with early terrestrial plants (Pirozynski and Malloch 1975; Prasad et al. 2017). Plants are almost universally populated by endophytic and mycorrhizal fungi (fungi that colonize the host plant's root system), by bacterial biofilms on plant surfaces, bacteria living inside plant tissues as endophytes, nitrogen fixing

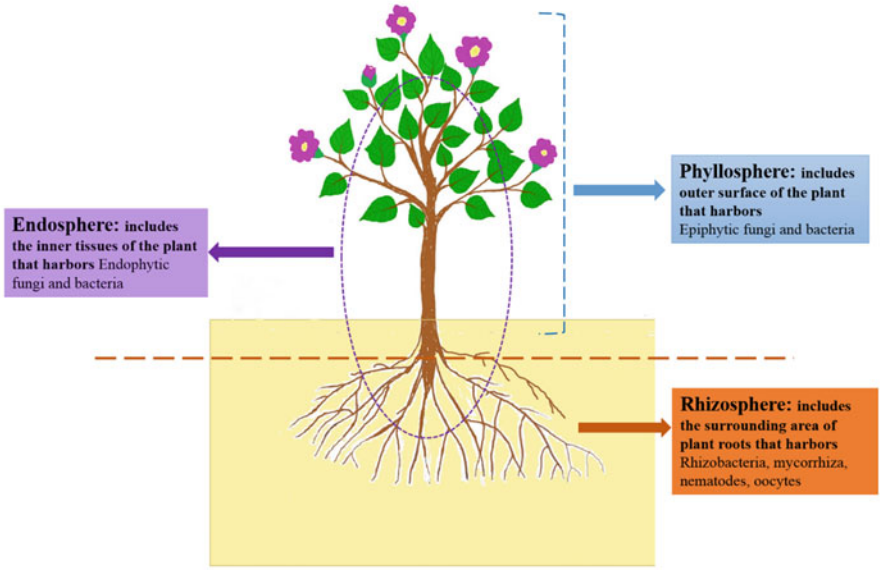


Fig. 24.1 Location of beneficial microorganisms in plants that promote growth and development. A plant is now defined as a metaorganism, comprising of numerous microorganisms closely associated in specific locations. The microbes are classified according to the sites they inhabit. The outer surface of a plant termed phyllosphere is the habitat for fungi and bacteria which are known as epiphytes; they are mostly air borne organisms. The endosphere includes the inner tissues of both roots and shoots. These parts harbor intercellular and intracellular endophytic microorganisms. Rhizosphere, the complex area surrounding the roots is deeply influenced by the same and contains bacteria and fungi that form close association with plant roots

bacteria colonized inside root or stem nodules, and many pathogenic microorganisms developing infections on leaves, roots and stems. There is ample substantiation that microbes influence plant health directly or indirectly by their effects on functional traits like nutrient delivery, photosynthetic changes, plant development and stress tolerance (Friesen et al. 2011; van der Heijden et al. 2008).

This chapter is concentrated on understanding the molecular dialogue used in transkingdom communication between plant and microbes that help to mediate plant growth and development. The chapter will describe some of these microorganisms and the various key players involved in the processes of interactive attachment with plants.

24.2 Plant–Microbe Cross-Talk in the Rhizosphere

The rhizosphere is a complex ecosystem, formed around the soil that is in close contact with plant roots. It is a crossroad for nutrient exchange between plants and soil microbes, and this exchange is most likely mediated by density-dependent

bacterial signaling, or quorum sensing (QS), between and among plants and bacteria living at high cell densities in the rhizospheric soil (Vitousek and Howarth 1991). The combined genome of this microbial community is much larger than that of the plant and is also referred to as the plant's second genome.

The substances released by plant roots are collectively termed "rhizodeposits." These compounds include (i) water-soluble ions and low-molecular-mass compounds such as mono-saccharides, amino acids and organic acids which are lost passively along a concentration gradient, (ii) high molecular-mass compounds such as carbohydrates, proteins and lipids which are actively transported along an electrochemical gradient, (iii) insoluble mucilage composed of polysaccharides and poly-galacturonic acid, (iv) an array of secondary metabolites such as antimicrobial compounds, nematicides and flavonoids and (v) remnants of the dead and lysed root-cap and border cells (Hale et al. 1978; Whipps 1990).

Of these, sugars and amino acids are thought to be released in the greatest quantities. This release or exudation of a large assortment of chemicals in the rhizosphere comes at a significant cost of carbon and nitrogen to the plant, with the ultimate benefit of attracting and promoting beneficial microorganisms while combating pathogenic or otherwise harmful ones (Hartmann et al. 2014). It is estimated that between 20 and 40% of all photosynthetically fixed carbon is eventually transferred to the rhizosphere. A plant bears this high cost because of the significant influence that the rhizosphere exerts on its health by affecting processes such as nutrient and water uptake and establishment of beneficial interactions with soil microbial populations.

The plant–microbe interaction in the rhizosphere may be categorized as associative, symbiotic, neutralistic, or parasitic. The positive interactions include symbiotic and associative interactions with beneficial microbes, such as endo- and ectomycorrhizal fungi, nitrogen-fixing bacteria and plant growth-promoting rhizobacteria (PGPR) whereas negative interactions include association with parasitic plants, pathogenic bacteria, fungi, oomycetes, nematodes and invertebrate herbivores (Haldar and Sengupta 2015; Prasad et al. 2015).

The most understandable rhizobial microorganisms' interaction with plants are the interactions between N_2 fixing bacteria and the arbuscular mycorrhizal fungi or AMF (both of them are therefore discussed separately below) which facilitate plant nutrition through the acquisition of nitrogen and phosphorus, respectively, in exchange for fixed carbon. Recent studies have shown striking similarities between rhizobia and AMF in their crosstalk with the host plant. Non-symbiotic microorganisms can also facilitate the uptake of nutrients and trace elements. For example, bacteria and fungi can provide iron to plants *via* siderophores, such as the pyoverdines produced by fluorescent *Pseudomonas* species and rhizoferrin, produced by *Rhizopus arrhizus* (Yehuda et al. 2000; Das et al. 2007) which are not commonly known as symbionts. It has been shown that the insect-pathogenic fungus *Metarhizium robertsii*, which is a plant endophyte as well, can transfer nitrogen from the insect to the plant through a tripartite interaction (Behie et al. 2012). Interactions between bacteria and AMF can also be beneficial such that the bacteria may help establish the mycorrhizal symbiosis, although the mechanisms

involved are still unclear. Reciprocally, AMF can have an impact on bacterial colonization and diversity (Philippot et al. 2013). A plethora of studies indicate how below-ground interactions can influence above-ground communities of herbivores, carnivores, mutualists and symbionts that interact with plants and vice versa. Plant response to above-ground herbivory might depend on the association of the plant with mycorrhizal fungi. Reciprocally, defense responses that are induced in the phyllosphere can spread systemically to the roots and affect the rhizosphere microbiota (Philippot et al. 2013).

24.3 Symbiotic Signaling in Nitrogen Fixation

Biological nitrogen fixation is a process that can only be performed by certain prokaryotes. In some cases, such bacteria are able to fix nitrogen in a symbiotic relationship with plants. Bacteria of the genera *Azorhizobium*, *Bradyrhizobium, Mesorhizobium, Rhizobium*, and *Sinorhizobium* (collectively referred to as *Rhizobium* or rhizobia) are able to establish an endosymbiotic association with legumes. They produce nodules on the roots of legume plants, inside of which they transform N_2 of the atmosphere into NH_3 that can be used by plants. In return, plants provide the bacteria with the carbon compounds that they release (Gyaneshwar et al. 2011; Oldroyd et al. 2011). It has been shown that bacteria in stems of sugarcane (Velázquez et al. 2008), residing in the apoplast (the space outside the plasma membrane through which substances can diffuse freely) in a low nitrogen, high-sucrose environment can have N_2 fixing ability.

The very complex process of nodule formation starts with some intricate communication between the plant and the microorganisms. The formation of a nodule requires the reprogramming of differentiated root cells to form a primordium, from which a nodule can develop. When rhizobia have colonized the root surface of their host, they induce morphological changes in the epidermis. These morphological changes are preceded by the induction of certain genes in a broad region of the epidermis. The best-studied examples are the early-nodulin genes, ENOD12 and ENOD11 (Scheres et al. 1990; Journet et al. 2001). In some root hairs, the rhizobia induce deformations that resemble a so-called shepherd's crook, and such curled root hairs play an important role in the infection process. During the curling process, the bacteria become entrapped in the pocket of the curl. There the plant cell wall is modified in a very local manner, the plasma membrane invaginates, and new plant material is deposited. In this way a tube-like structure, the infection thread, is formed that contains the bacteria. The infection thread will grow towards the base of the root hair cell and subsequently to the nodule primordium (Fig. 24.2) (Brewin 1998). Upon release, the bacteria remain surrounded by a membrane of plant origin and subsequently will differentiate into their symbiotic form and will start to fix nitrogen.

Legume-Rhizobia interactions are mediated by host-specific flavonoids (2-phenyl-1,4-benzopyrone derivatives) secreted in the root-exudates. These molecules

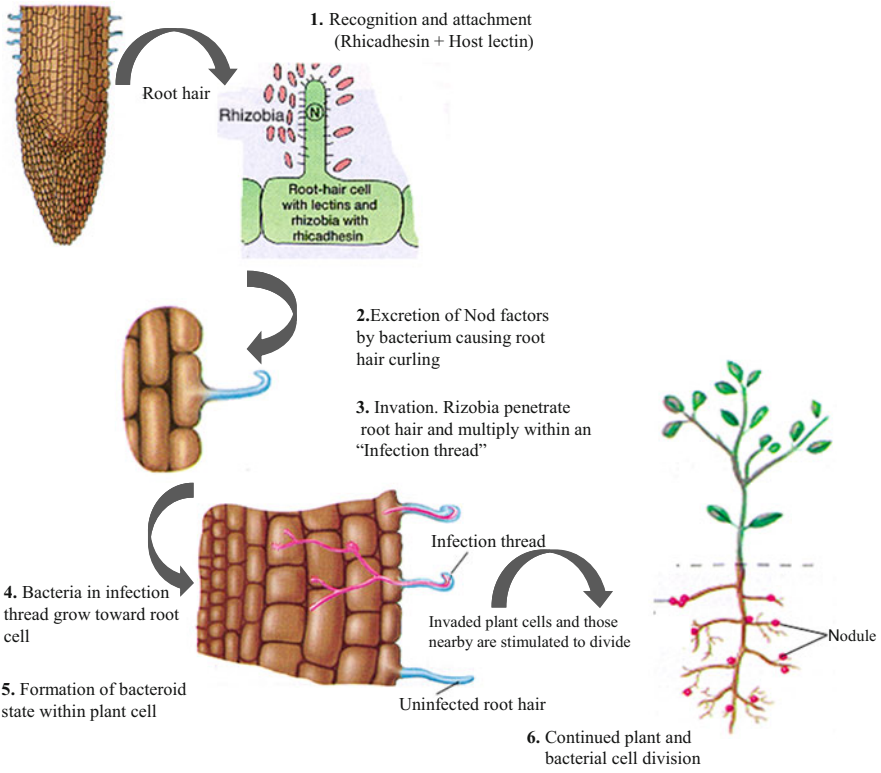


Fig. 24.2 Symbiotic root-nodule development. Root nodule formation is the key step of symbiosis development between legume plants and nitrogen fixing bacteria. Nodules are specific swelled structural features of legume plants that provide an ideal oxygen limiting condition for fixing nitrogen by bacteria populating the nodule. The development of nodules begins with attachment of bacteria to an outer layer of plant root upon recognition by plant signal (lectins) followed by morphological changes in root epidermis (*1*). This is perceived by some precise plant gene expressions and the plant signals induce the bacteria to produce some specific factors (Nod factors) that in turn are recognized by plants stimulating root hair curling (*2*). The curled root hairs entrap the bacteria as they start to populate the hair threads (*3*) and upon stimulation by Nod factors the curled root hairs start cell division and development (*4*). This developmental cascade forms nodule primordial (*5*) and ultimately multiple cell division processes result in nodule formation. The development of a nodule requires the reprogramming of differentiated root cells to form a primordium, from which a nodule can be produced (*6*)

belong to a diverse family of aromatic compounds derived from a plant's secondary metabolism. Depending on the host and the bacterium, flavonoids activate a series of transcriptional events culminating in the production of main rhizobial nodulation signals called *Nod* factors (NF) or lipo-chito oligosaccharides (LCOs) (Fig. 24.3) (Spaink 2000).

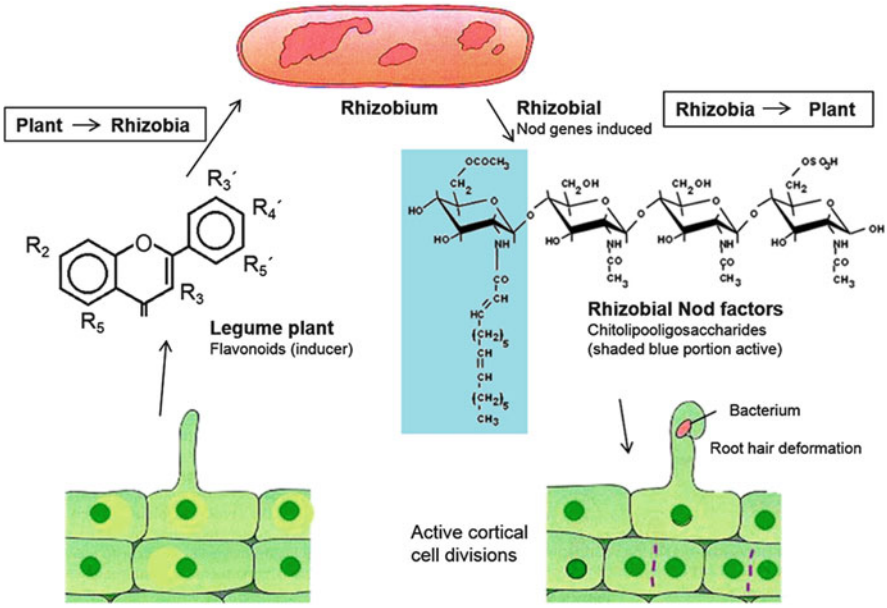


Fig. 24.3 Molecular communication between rhizobial and legume symbionts. Molecular signalling is key to the development of a nodule and the resultant symbiosis of legume-N_2 fixing bacteria. These are specific communications that render legume and related plants the explicit capability. The legumes secrete flavonoid compounds around their root. These flavonoids induce rhizobia to produce Nod factors or lipochitooligosaccharides (LCO). The different structures of the Nod factors provide definite interactions between specific Rhizobia species and legume plants. These factors are recognized by plants and prompt different cascades of modulated development in them which eventually allow attachment of the bacteria to plant root hair cells

These LCOs are recognized by plant receptor kinases at the root epidermis, thereby activating a well-characterized signal cascade leading to nodule formation (Oldroyd et al. 2011). The non-reducing terminal sugar of the LCO is attached to an *N*-acyl group, and this acylated LCO can be further modified by a variety of substituents on the GlcNAc subunits, such as methyl, fucosyl, acetyl and sulphate groups (Denarie et al. 1996). These residues, coupled with the length and degree of saturation of the *N*-acyl group, vary widely between Nod factors produced by different rhizobial species, and this has been shown to be important for the specificity of interactions between different rhizobia and host legume species (Roche et al. 1991).

Mutant analyses in a range of leguminous species have defined loci that are essential for the recognition of *Nod* factors and the activation of all known *Nod* factor-mediated responses (Denarie et al. 1996). Genes at these loci are found to encode receptor-like kinases with LysM (lysine motif) domains in the extracellular region. These domains bind to oligosaccharides, the major composition of bacterial cell wall. Two forms of LysM receptor-like kinases have been shown to function in

Nod factor signaling: (i) LysM I clade and (ii) LysM II clade. Recently it was shown that these receptors bind Nod factors at nanomolar-range concentrations (Ruyter-Spira et al. 2013). Such a binding affinity is consistent with the concentration of *Nod* factors required for the activation of symbiotic signaling processes, such as calcium oscillation. Both receptor types are equally important for rhizobial colonization and have been shown to interact *in planta*. LysM I type has a functional kinase domain that is essential for *Nod* factor signaling and is capable of promoting auto-phosphorylation, as well as trans-phosphorylation of the LysM II type's cytoplasmic domain. This suggests that these receptor-like kinases function as heterodimers or hetero-complexes and indicates that trans-phosphorylation events between the different kinase domains might be important for the activation of downstream signaling. Leucine rich repeat (LRR) domain is evolutionarily conserved in many proteins associated with innate immunity in plants, invertebrates and vertebrates, and are known to be involved in microbial interactions with plants. An LRR-containing receptor-like kinase gene known as symbiosis receptor-like kinase (*SYMRK*) in *L. japonicus* and *DMI2* (also known as *NORK*) in *M. truncatula* is required for Nod factor signaling (Ruyter-Spira et al. 2013). The exact functions of *SYMRK* and *DMI2* have not yet been defined, but it is reasonable to presume that the encoded proteins act as co-receptors with the LysM receptor complex during Nod factor signaling. Nucleus-associated oscillations in calcium, link *Nod* factors to gene regulation in plants. Although the mechanisms that link the receptor activation to calcium oscillations in the nucleus remain unclear, it is reasonable to presume that the receptor complex activates a second messenger that regulates calcium channels. Two cation channels located on the nuclear membranes, CASTOR and POLLUX, have been identified as essential for symbiotic calcium oscillations in *L. japonicus*. Detailed calcium imaging combined with the location of POLLUX and CASTOR implies that the symbiotic calcium store is the endoplasmic reticulum (Capoen et al. 2011) and that nuclear release from this store is controlled by the nuclear envelope channels. Calcium oscillations are primarily sensed by the nuclear-localized protein, calcium-and calmodulin-dependent serine/threonine protein kinase (CCaMK). Stimulated CCaMK replaces the activity of upstream components, implying that the symbiotic calcium oscillations happen solely to activate CCaMK (Fig. 24.4). Activated CCaMK dictate the nature of further gene expression ensuring nodulation signaling.

Even though a host plant benefits from nitrogen fixed by nodule forming microorganisms, excessive nodulation has been found to have a negative impact on plant growth since nitrogen fixation is energy intensive. To overcome this, legume plants have evolved a negative feedback route which checks for the correct number of nodules in a plant. This is an autoregulatory pathway (AON for autoregulation of nodulation), involving long distance signaling between the roots and shoots of a plant. This allows for a correct symbiotic balance. Studies based on *Lotus japonicus*, a leguminous plant have identified two leucine-rich repeat receptor-like kinases, hypernodulation aberrant root formation (HAR1) and KLAVIER. In the plant shoot, these kinases monitor the formation of the signaling molecules, CLE-RS1/2 peptides and allow for the production of SDI, a shoot

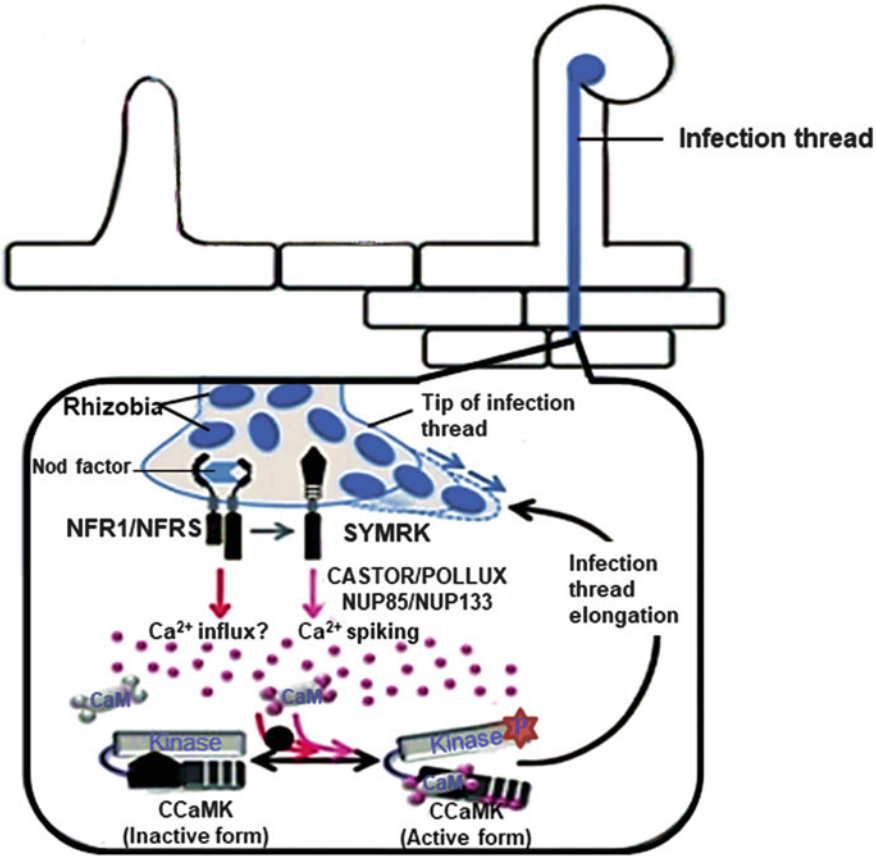


Fig. 24.4 *Nod*-factor signalling pathway in legumes. Nod factors are produced by Rhizobia upon stimulation by plant signals. These factors significantly modulate plant root development which ultimately give rise to nodules. These factors are primarily recognized by plant kinase receptors and is proposed to couple with the process of calcium signalling. Upon recognition of the nod factors these receptors (Nod Factor Receptors, NFRS) dimerize and together with the co-receptors like SYMRK they help activate the cation channels (e.g CASTOR and POLLUX) located in the outer nuclear membrane. Activation of these channels triggers calcium release from endoplasmic reticulum which shares membrane with the outer nuclear membrane and the calcium combines with calcium binding proteins (CaM) and activates calcium dependent protein kinase CCaMK which then dictates the downstream molecular process required for the development of symbiosis (adapted from Kouchi et al. 2010)

derived inhibitor that travels to the root to signal a block to further nodule formation (Capoen et al. 2011). Sasaki et al. (2014) have shown that CLE-RS1/2–HAR1 signaling leads to the synthesis of cytokinins which possess the inhibitor activity that meticulously suppresses the formation of nodules.

24.4 Plant Signaling in Arbuscular Mycorrhizal Association

The arbuscular mycorrhizal (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 million years ago (Oldroyd 2013; Prasad et al. 2017). The AM fungi are obligate biotrophs and depend entirely on plants to provide them with carbon. Their main contribution is to assist plants with the acquisition of mineral nutrients, particularly phosphorus, and recently it has been suggested that in an AM symbiosis, plants receive all of their phosphorus *via* their fungal symbiont. Soil phosphate is transported along the mycelial network into the inner cortex of the root, where it is delivered at specialized fungal structures called arbuscules that also serve for the sugar uptake from the plant. Arbuscules are tree-like structures formed by profuse dichotomous hyphal branching in the lumen of cortical cells surrounded by a plant derived plasma membrane called the periarbuscular membrane (PAM). The PAM is very special as it has a different protein composition from the rest of the plasma membrane, hosting the transporters that will be key in the nutrient exchange with the fungal partner (Pumplin and Harrison 2009).

Despite the ecological importance, the molecular and genetic mechanisms of the signaling underlying this symbiosis are only partially understood. Interestingly, LCO signals are also involved in symbiotic associations of plants and arbuscular mycorrhizal fungi. These chemicals are most probably produced continuously, in order to establish long-lasting colonization, but apparently is not very specific because there is no plant host specificity. This indicates that either the plant signals are conserved throughout the plant kingdom or possibly that a broad range of plant compounds are involved (Harrison 2005).

AM fungal spores can germinate in water, which indicates that they do not require a plant signal for germination; however, plant root exudates and volatiles, including CO_2, stimulate germination, which suggests that the fungus can sense components of the rhizosphere (Philippot et al. 2013). In the absence of a plant root, hyphal growth will cease but this happens before spore reserves are depleted so that the fungus has an opportunity to germinate again and additional chances to find a host root. Some species, with particularly large spores, are capable of germinating up to 10 times.

Fungi perceive a signal in root exudates that triggers an increase in respiration and the onset of active growth. As fungal growth in the presence of exudates is extensive and continues until spore reserves are depleted, it has been proposed that the signal in the exudates initiates a transition to a "pre-symbiotic growth phase," at which point a fungus is committed to full utilization of its spore reserves. Some molecules secreted from plant roots act as signals for AM fungi (D'Haeze et al. 1998); an example of which is strigolactone, a new class of plant hormones of increasing importance in plant biology. Root colonization by AMF involves the strigolactones which serve as rhizospheric signals to activate responses in AMF, including hyphal branching, and thereby promoting mycorrhizal colonization of the root surface. This involves the fungus making contact with the plant epidermal cell

wall and forming a hyphopodium which is a lobed hyphal contact point with the root that serves as the entry point of the fungus into the epidermis. The hyphopodium is a specialized structure similar to, but distinct from, the pathogenic appressorium, which is the tip of a hyphal branch that facilitates penetration of the host plant (Jeremy et al. 2013). The fungus then passes through an epidermal cell and colonizes the root cortex by extensive intercellular hyphal growth and the formation of terminal intracellular structures called arbuscules. The arbuscule, which serves as the nutrient exchange interface in the symbiosis, is highly branched and is surrounded by plant plasma membrane.

Recently, monomers of cutin (Harrison 2005), the biopolyester derived from cellular lipids have been implicated as a specific class of plant signaling factors which play a crucial role in AMF stimulation. Two loci, RAM1 and RAM2 ('required for arbuscular mycorrhization'), have been identified in *Medicago truncatula* mutants seriously affected in AM symbiosis; they encode a GRAS domain transcription factor and an acyl transferase, respectively, involved in the production of cutin monomers (Fig. 24.5). This provides the first insight into a

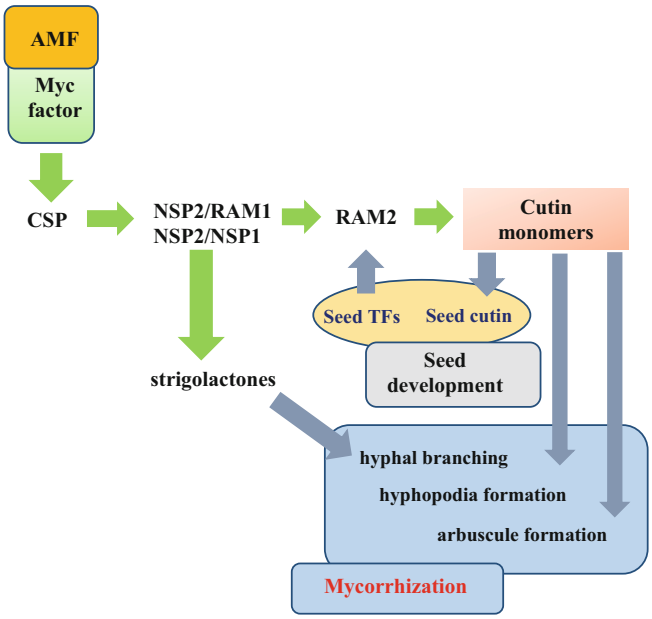


Fig. 24.5 AMF-plant signalling and role of RAM1 and RAM2 in cutin monomer biosynthesis. The arbuscular mycorrhizal fungi (AMF) develops symbiotic relationship with vascular plants. Precise molecular signalling between plant and fungi develops this friendly interdependent relationship. The fungi produces some factors which are recognized by plants through common symbiosis pathway (CSP). This pathway in turn activates GRAS transcription factors NSP1, NSP2, RAM1 and acyl transferase RAM2. These plant factors in turn induce production of the plant hormone, stringolactone and also are associated with cutin monomer production. Both these products help in plant and fungal development as well as arbuscular structure formation leading to the development of mycorrhizal symbiosis

mycorrhization specific signaling pathway and reveals cutin monomers as a critical component of signaling in mycorrhizal symbiosis.

24.5 Plant Signaling to Endophytes and Epiphytes

The concept of plant "outsourcing" it's essential components for efficient growth and development to symbiotic microorganisms has further become plausible with the documentation of the phyllospheric (air-contact surface of plants) and endospheric (inner parts of plants) microorganisms. Plant development is now considered a result of interspecies communication (Gilbert et al. 2012). The useful traits of non-pathogenic endophytes and epiphytes of plants promote the potential construction of microbiomes for crops to enhance agronomy with reduced chemical inputs and at cheaper cost. Nonetheless, to combine useful microbiomes we need at first to understand how they function.

The phyllosphere of a plant includes the aerial parts of the same, predominantly the leaves, nonetheless also comprises of surfaces of stems (caulosphere), flowers (anthosphere), fruits (carposphere), and leaves (phylloplane), all of which can harbor different microbial inhabitants (Junker et al. 2011). These microbial communities can range from pathogens to symbionts (Lindow and Brandl 2003). Epiphytes are non-pathogenic fungi, bacteria, or algae that persist on the plant surface without interior infiltration at any point in their life cycle (Zambell and White 2015). An epiphytic lifestyle predominates for commensal phyllosphere microorganisms. From several studies, it is apparent that plants actively select their epiphytic organisms and therefore their distribution is not random. While environmental factors largely affect the microbial epiphytic community, findings suggest the involvement of plant genetic features that recruit these microbes, in a selective manner. Conceivable mechanisms for such effects is initiated likely by the chemistry of leave surfaces (Hunter et al. 2010), jasmonic acid (Kniskern et al. 2007) or GABA (γ-aminobutyric acid) signaling pathway) (Balint-Kurti et al. 2010).

Plants emit volatile organic compounds (VOCs) including terpenes, phenylpropanoids, benzenoids, nitrogen and sulfur containing compounds that have a pertinent role in defining the features of the microbial population that dwell on plant exteriors, through their antimicrobial properties (thereby inhibiting growth of microorganisms) or as carbon sources for some microorganisms (stimulating inhabitants). VOCs are efficient mediators for communication acting universally as attractants, repellents or warning signals in organisms from all kingdoms (Ortíz-Castro et al. 2009). These volatile compounds are actively produced and used as a refined "language" by plants to pursue communication with other organisms (Choudhary et al. 2008). Soil microbial activity can degrade plant produced monoterpenes and bacteria like *Psuedomonas flourescens* and *Alcaligenes xylosoxidans* have been shown to metabolize α-pinene, a bicyclic monoterpene and use the same as the only source of carbon. Plant produced sesquiterpenes found

in the roots are also known to serve as carbon sources for microorganisms associated with roots of some plants (Junker and Tholl 2013). On the other hand, surface microorganisms located between plant-atmosphere contacts (the interface for essential gaseous exchange related to plant growth, for example, CO_2 for carbohydrate production) are able to modify plant physiology and biochemistry (Farré-Armengol et al. 2016). Other plant VOCs like methanol, some short chain oxygenated compounds and low molecular weight fatty acid derivatives are also active in communicating with microbes. Methanol (MeOH) emitted from wounded plants have been found to retard the growth of a bacterial pathogen, *Ralstonia solanacearum* in plants within the neighborhood known as "receiver" plants (Dorokhov et al. 2012).

Endophytes (typically bacteria or fungi) are non-pathogenic towards their hosts and spend at least some point in their life cycle by colonizing the interior spaces of plant tissues, including roots, stems, leaves, flowers or seeds (Hallmann et al. 2006). They may be limited in their distribution and metabolic activities within specific plant tissues, or can exist in tissues in a nearly dormant phase; or they could possibly be circulated through multiple tissues of host plants (Rodriguez et al. 2009). In terms of plant cell and tissue sites, endophytes are principally intercellular, in many cases, may become intracellular and enter into the host plant cytoplasm or become localized in the periplasmic area, between the cell wall and plasma membrane (Paungfoo-Lonhienne et al. 2010; Thomas and Sekhar 2014).

Different endophytes are known to colonize different parts of a plant. The reason behind such specificity must depend on the definite plant metabolites produced locally. After initial colonization, some endophytes can move to other areas of the plant by entering the vascular tissues and spreading systemically (Gaiero et al. 2013). Studies have shown a considerable distribution pattern of endophytes within plants; however the mechanisms underlying the distribution pattern is still unclear and is of intense research interest. Endophytic bacteria can be obligates which are unable to survive outside the plant tissues and are considered to be transmitted via seeds, as opposed to the facultative endophytes which originate from soil as free living bacteria and colonize plants when they get the chance to enter through coordinated infection (Hardoim et al. 2008). This has obvious benefits of specialized service provided by the endophytes which the plants enjoy in return.

Root endophytes reside in a vast number of plant species as part of their root microbiome, with some found to influence positively the growth of a plant including increase in root length, shoot biomass and root fresh weight. Such endophytes colonize and infiltrate the epidermis at sites of lateral root development, below the root hair zone, and in root fissures. Chemotactic response is the key for root colonization. Some microbes penetrate the root by outnumbering other microbial species. This requires coordinated gene expression of specific proteins in order to invade the plant tissue, fight the immune system of the host plant, and find shelter in a niche within the plant. Coordinated invasion by microbes on the root surface comprises many signaling pathways and mutual signaling between host and endophytes and between endophytes (Morris and Monier 2003; Rosenblueth and Martínez-Romero 2006).

Endophytic fungi are more known to protect plants against pathogenic attack, by producing several anti-pathogenic secondary metabolites. These compounds comprise of alkaloids, steroids, terpenoids, peptides, polyketones, flavonoids, quinols, phenols, and chlorinated compounds (Hardoim et al. 2015). These metabolites prompt a plant's induced systemic resistance (ISR), thereby triggering its own defense army—by influencing the expression of pathogen related (PR) genes, VOCs etc. Such signals protect a plant significantly from insects, nematodes and bacterial pathogens. The enigmatic feature is that, in many cases, a plant's defense machinery is triggered even in the absence of mycotoxins. This may happen due to the induction of other plant traits that may alter their nutrition/metabolic state (Schulz 2006).

Several plant-associated bacterial species use Quorum Sensing (QS)—a process depending on population size—for their establishment within a plant—and adjust a wide range of phenotypes including rhizosphere competence, virulence, conjugation, secretion of hydrolytic enzymes, and the production of secondary metabolites (Newton and Fray 2004). The most common QS system in gram-negative bacteria uses acetyl homoserine lactone (AHL) as signals. The typical AHL QS system contains a LuxI family synthase synthesizing the AHL. In each bacterial cell, AHL works at a low basal level and also diffuses through the membrane as an amphophilic substance to the neighborhood. Hence it is the AHL concentration that denotes the cell density. When bacterial cell-density reaches a specific threshold ("quorum"), AHL molecules bind to LuxR receptor protein in the bacterial cytoplasm. This AHL-receptor complex acts as a transcriptional regulator. Thus, the response to the AHL-signaling compounds is auto-inducing, making this regulation extremely sensitive (Fuqua et al. 2001). Recently it has been demonstrated that, AHLs can also act as interkingdom signals influencing plant gene expression (Venturi and Fuqua 2013). Some compounds that are plant derived have been reported to impede QS and play the role of agonists or antagonists of bacterial QS systems (Bauer and Mathesius 2004).

A remarkable discovery has shown that some plant-associated bacterial LuxRs do not bind and thus do not respond to AHL mediated compounds but they react against low molecular weight plant-derived molecules (Bauer and Mathesius 2004). These luxRs are now defined as a new subfamily of proteins which have evolved away from binding to AHLs but can respond to plant signals, thus representing an extensive novel inter-kingdom signaling module. In the pseudomonads of the rhizosphere, one of these proteins called PsoR was found to be involved in transcriptional regulation by responding to various plant compound(s) which exert antimicrobial activity (Subramoni et al. 2011). In this widespread interkingdom signaling system, the crucial plant signal(s) are the low molecular weight plant secondary metabolite(s) which direct this communication. Plants alter their gene expression, modify their protein profile and adjust their development, in response to the presence of AHL in their surroundings (Bauer and Mathesius 2004). Whether AHLs induce systemic responses or are transported within plants seem to depend on the structure of the AHL molecule, in particular on the length and residues of the fatty acid chain (Schenk et al. 2012). Developmental

changes altered by AHL that are related to changes in cell division, cell elongation and cell differentiation and its mechanism of action are found to be independent of auxin signaling. Multiple studies have shown that plants discharge components that precisely stimulate or inhibit AHL dependent QS responses through interactions with microbial AHL receptors (Teplitski et al. 2000); These QS imitators target different steps of the QS circuit, including signal synthesis, signal stability and signal sensing (Teplitski et al. 2000). Specific flavonoids are released that enable certain bacteria to specifically populate a host plant through coordinated gene regulation required for colonization.

There are ample evidences of plants producing quorum-sensing inhibitors (Gaiero et al. 2013). These quenching compounds are considered to serve as an armor against quorum sensing pathogenic species. Mycotoxins such as fusaric acid, penicillic acid, and patulin are now viewed as quorum quenching compounds produced by various fungi which can disrupt quorum-sensing regulation induced by pathogenic bacteria (Rasmussen and Givskov 2006).

24.6 Microbial Signaling to Plants

Plant growth promoting bacteria and fungi, nodule forming rhizobia and arbuscular mycorrhiza, are invariably recognized as non-self by the plant, which uses committed pattern recognition receptors (PRRs) to perceive conserved microbe-specific molecules, termed microbe-associated molecular patterns (MAMPs) such as lipopolysaccharides, peptidoglycans, flagellin, and chitin (Zamioudis and Pieterse 2012; Millet et al. 2010). The MAMPs trigger a local basal immune defense, which can then be converted into systemic defense responses that are controlled by regulatory networks connecting signaling *via* the plant hormones, salicylic acid, jasmonic acid, ethylene, and others (Venturi and Keel 2016). Plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) generally induce plant defense response which is known as induced systemic resistance (ISR) (Zamioudis and Pieterse 2012). This defense signaling depends on jasmonic acid and ethylene, and is separate from the systemic acquired resistance (SAR) which is primarily induced by pathogens and involves salicylic acid signaling (Ryals et al. 1996). The immune response triggered by beneficial microorganisms is relatively minor and is based on a process called priming which prepares the plant, upon sensitization by the microbes, to react more efficiently to abiotic and biotic stress such as attack by leaf pathogens and pests (Venturi and Keel 2016). Beneficial rhizosphere microorganisms counter immune recognitions; the signaling involved in these immune interactions is little understood and has generated considerable interest in these microorganisms in recent years, and research in this field is rapidly evolving (Zamioudis and Pieterse 2012).

Rhizospheric microorganisms elicit plant responses not only *via* MAMPs and effector proteins but they also do so *via* diverse signaling molecules. Mycorrhiza produce small secreted proteins (SSPs) that act as mutualistic effectors promoting

mycorrhization by altering hormonal signaling pathways in their plant host (Plett and Martin 2015). The ectomycorrhizal fungus—those which can penetrate the root but cannot invade the cell of host plants, release upon root contact a small protein from its hyphae. This protein then enters the host cells, localizes to the nucleus, and interacts with plant hormone co-receptors to counteract jasmonic acid signaling and promote symbiosis (Teplitski et al. 2000).

Antimicrobial compounds are another class of microbial molecules that stimulate systemic plant responses. 2,4-diacetylphloroglucinol (DAPG) induces salicylic acid and ethylene signaling-dependent ISR against fungal and bacterial leaf pathogens (Iavicoli et al. 2003). DAPG is also described to have positive effects in root development, and this is apparently found to occur *via* an auxin-dependent signaling pathway (Brazelton et al. 2008).

Many VOCs which act as microbial signaling molecules are of considerable interest because of their influence on plant growth promotion or inhibition (Bailly and Weisskopf 2012). Bacterial volatiles have been found to be more complex than that of plants. Indole, another bacterial signaling VOC produced by various PGPRs affects root development *via* the auxin signaling pathway (Bailly et al. 2014). Interestingly, indole released by plants also functions as a potent volatile signal that primes the producer and neighboring plants against attacks from herbivorous insects. Similar to AHLs and antibiotics, VOCs can also have multiple biological roles in intra- and interspecies interactions among the bacteria themselves. There are numerous reports showing that volatiles produced by bacteria such as ammonia, butyrolactonas, HCN, phenazine-1-carboxylic acid, alcohols, among others, may have activity *in vivo* (Trivedi and Pandey 2008). Production of bioactive VOCs is widespread and highly diverse among PGPR and PGPF. It is evident that PGPR VOCs can trigger different hormonal signaling networks in plants, involving cytokinins, brassinosteroids, auxin, salicylic acid and gibberellins. This has generated new expectations about the role of volatiles during plant–microorganism relationship with regard to plant development (Ortíz-Castro et al. 2009). Experiments on *Arabidopsis* suggest that VOCs have direct implications on auxin production as seen by microarray analysis and are therefore directly related to plant morphogenesis. Also, another study has shown that VOCs from plant associated microorganisms can modulate endogenous ABA (abscisic acid)/sugar signaling and improve the photosynthetic efficiency of plants. VOC-mediated microbe–plant interactions are expected to be by far more complex than uncovered so far. A further class of microbial molecules with interkingdom signaling properties are phytohormone-like compounds, including auxins, gibberellins, and cytokinins, that are not only produced by PGPR and PGPF but also by bacterial and fungal pathogens. They affect growth, organ development, immune responses, and hormonal signaling in plants (Fig. 24.6).

Numerous evidences are suggestive that growth promoting phytohormones produced in abundance by plant associated microbes regulate plant growth and development. Diverse bacterial species produce auxins and their precursors as part of their metabolism. Auxins are quantitatively the most abundant phytohormones secreted by *Azospirillum* species, and it is generally agreed that auxin production is

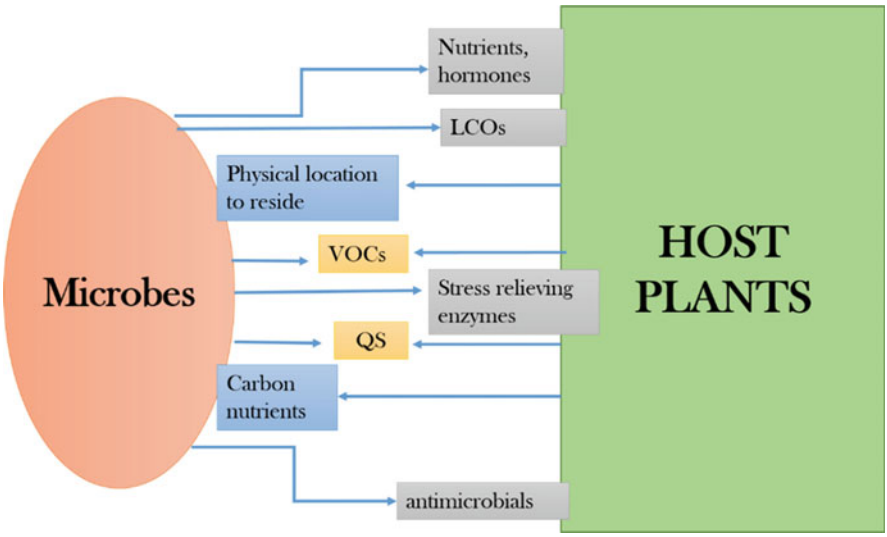


Fig. 24.6 The key molecules that have a role in interkingdom signaling between plants and its associated microbes. Plants actively recruit microbes by the secretion of volatile compounds and other chemicals and provide them with carbon sources as well as a secure physical location for them to inhabit. In exchange the microbes provide with nutrients that are otherwise unreachable for plants as well as hormones for favorable growth of plants. They also provide antimicrobials to protect the plants against pathogens and relieve stress through abiotic stress alleviating hormones. The interplay between them are mediated by the volatile organic compounds (VOCs), molecules involved in quorum sensing (QS) and other specific genetic traits

the major factor responsible for the stimulation of root system development and growth promotion by this bacterium (Ortíz-Castro et al. 2009). Many fungal species also produce auxins. Recent findings on the role of fungal-produced indole acetic acid (IAA) in different plant–fungus interacting systems open the possibility that fungi may use IAA and related compounds to interact with plants as part of its colonization strategy, leading to plant growth stimulation and (Ortíz-Castro et al. 2009) modification of basal plant defense mechanisms. Cytokinins, produced by plant associated microorganisms have been implicated in an increase in growth of plants (Garcia de Salamone et al. 2001). In *Arabidopsis*, cytokinins produced by bacterial species associated with a plant were found to promote biomass growth. Disruption of cytokinin receptors result in significant reduction of growth promotion activity, highlighting the importance of cytokinin perception in a plant's response to microorganisms. Endophytes have evolved ways to use plant hormone signaling pathways to their advantage. ACC deaminase is a bacterial enzyme that is often associated with alleviation of plant stress. This enzyme is responsible for lowering the levels of ethylene in a plant by cleaving the plant-produced ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) to ammonia and 2-oxobutanoate, preventing ethylene signaling (Hardoim et al. 2015). The plant hormone ethylene acts in germination of seeds and responds to various stresses, and

it is the key regulator of colonization of plant tissues by bacteria. This suggests that, apart from stress alleviation, ACC deaminase supports colonization of a number of bacterial endophytes. Preferential selection of plant growth promoting bacteria with high ACC-deaminase activity by plants could benefit the plant and give ACC-deaminase containing endophytes an advantage. Endophytes use ACC-deaminase and indole acetic acid to direct their hosts to effectively reprogram some of their signaling pathways (Gaiero et al. 2013).

A study between interaction of plants and endofungal species (Tanaka et al. 2006) have shown that reactive oxygen species (ROS) produced by the fungi act as signals for mutualism, while mutation of the ROS producing traits shifts the interaction towards pathogenic consequences.

With the advent of more signaling related studies, it is expected that more players will be uncovered and the pathways will gradually be better understood.

24.7 Concluding Remarks

An abundance of focused research has revealed an intimate symbiotic relationship between plants and their associated microorganisms. Such associations leading to the formation of plant specific microbiomes are extremely diverse both at the structural and the functional levels. These studies have led to a fundamental change in our understanding of plant physiology. A high interplay of bacteria, archaea, fungi and protists forming the microbiome appear to be at work in shaping the physical and functional aspects of a plant. The plant microbiome is now recognized as an important entity of a plant and it has led us to consider plants as metaorganisms.

Climate change is intensifying the challenges faced in agriculture. It is causing the emergence of new phytopathogens the leading cause of crop yield decreases that is threatening food security. Environmentalists and in some cases policy makers are advocating use of natural means to combat such threats. Therefore for some time now we are noticing an increased use of biofertilizers and biopoesticides. However, there are questions regarding their efficiency and cost benefits. In this background our recent understanding of plant and microbe association and appreciation of the intricate crosstalk that takes place between them will help to develop means of harnessing the same for enhancing crop protection and sustainable improvement in its productivity. We now know that plant microorganism interaction can lead to significant changes in the metabolome and a concomitant change in its physiology and transcriptional status. An in-depth understanding of the same will allow us to use them in pathogen resistance and in general as bio-control agents. This chapter has focused on signaling between the host and its diverse and mostly benevolent guests and has illuminated how such communications can lead to growth enhancement including stimulus for germination, resistance from diseases and tolerance to stress. It is now apparent that signaling between plants and its microbiome can be used to engineer host microbial population of economically important crops in order to enhance productivity under conditions considered to be adverse for plant

growth. Adverse conditions ensuing from climate change will lead to more stressed conditions and such conditions are likely to become more frequent and severe in the near future. Therefore by a concerted manipulation of a plant microbiome the adversities of climate change such as increased incidence of plant diseases and decreased agricultural production can be thwarted to bring about a cleaner, greener and a more sustainable world.

References

Bailly A, Weisskopf L (2012) The modulating effect of bacterial volatiles on plant growth: current knowledge and future challenges. Plant Signal Behav 7:79–85

Bailly A, Groenhagen U, Schulz S, Geisler M, Eberl L et al (2014) The interkingdom volatile signal indole promotes root development by interfering with auxin signalling. Plant J 80:758–771

Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE (2010) Maize leaf epiphytic bacteria diversity patterns are genetically correlated with resistance to fungal pathogen infection. Mol Plant Microbe Interact 23:473–484

Bauer WD, Mathesius U (2004) Plant responses to bacterial quorum sensing signals. Curr Opin Plant Biol 7:429–433

Behie S, Zelisko P, Bidochka M (2012) Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science 336:1576–1577

Brazelton JN, Pfeufer EE, Sweat TA, Gardener BBM, Coenen C (2008) 2, 4-Diacetylphloroglucinol alters plant root development. Mol Plant Microbe Interact 21:1349–1358

Brewin NJ (1998) Tissue and cell invasion by Rhizobium: the structure and development of infection threads and symbiosomes. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Kluwer Academic Publishers, Dordrecht, pp 417–429

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M et al (2011) Nuclear membranes control symbiotic calcium signaling of legumes. Proc Natl Acad Sci USA 108:14348–14353

Choudhary DK, Johri BN, Prakash A (2008) Volatiles as priming agents that initiate plant growth and defence responses. Curr Sci 95:595–604

D'Haeze W, Gao M, De Rycke R, Van Montagu M, Engler G, Holsters M (1998) Roles for azorhizobial Nod factors and surface polysaccharide in intercellular invasion and nodule penetration respectively. Mol Plant Microbe Interact 11:999–1008

Das A, Prasad R, Srivastava A, Giang PH, Bhatnagar K, Varma A (2007) Fungal siderophores: structure, functions and regulations. In: Varma A, Chincholkar SB (eds) Microbial siderophores. Springer, Berlin, Heidelberg, pp 1–42

Denarie J, Debelle F, Prome J-C (1996) Rhizobium lipo-chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. Annu Rev Biochem 65:503–535

Dorokhov YL, Komarova TV, Petrunia IV, Frolova OY, Pozdyshev DV et al (2012) Airborne signals from a wounded leaf facilitate viral spreading and induce antibacterial resistance in neighboring plants. PLoS Pathog 8(4):e1002640

Farré-Armengol G, Filella I, Llusia J, Peñuelas J (2016) Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. Trends Plant Sci 21:854–860

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL et al (2011) Microbially mediated plant functional traits. Annu Rev Ecol Evol Syst 42:23–46

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS et al (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100:1738–1750

Garcia de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47:404–411

Gilbert SF, Sapp J, Tauber AI (2012) A symbiotic view of life: we have never been individuals. Q Rev Biol 87:325–341

Gyaneshwar P, Hirsch AM, Moulin L, Chen W-M, Elliott GN et al (2011) Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. Mol Plant Microbe Interact 24:1276–1288

Haldar S, Sengupta S (2015) Plant-microbe cross-talk in the Rhizosphere: insight and biotechnological potential. Open Microbiol J 31:1–7

Hale MG, Moore LD, Griffin GJ (1978) Root exudates and exudation. In: Elsevier Dommergues YR, Krupa SV (eds) Interactions between non-pathogenic soil microorganisms and plants. Elsevier, Amsterdam, pp 163–203

Hallmann J, Berg G, Schulz B (2006) Isolation procedures for endophytic microorganisms. In: Microbial root endophytes. Springer, Berlin, pp 299–319

Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471

Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Hartmann A, Rothballer M, Hense BA, Schröder P (2014) Bacterial quorum sensing compounds are important modulators of microbe-plant interactions. Front Plant Sci 5:131. https://doi.org/10.3389/fpls.2014.00131

Hunter PJ, Hand P, Pink D, Whipps JM, Bending GD (2010) Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (*Lactuca species*) phyllosphere. Appl Environ Microbiol 76:8117–8125

Iavicoli A, Boutet E, Buchala A, Métraux J-P (2003) Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. Mol Plant Microbe Interact 6:851–858

Jeremy DM, Donna RC, Kirsty JJ, Chengwu L (2013) Signaling at the root surface: the role of cutin monomers in mycorrhization. Mol Plant 6:1381–1383

Journet EP, El-Gachtouli N, Vernoud V, De Billy F, Pichon M, Dedieu A, Arnould C, Morandi D, Barker DG, Gianinazzi-Pearson V (2001) *Medicago truncatula* ENOD11: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. Mol Plant Microbe Interact 14:737–748

Junker RR, Tholl D (2013) Volatile organic compound mediated interactions at the plant-microbe interface. J Chem Ecol 39:810–825

Junker RR, Loewel C, Gross R, Dötterl S, Keller A et al (2011) Composition of epiphytic bacterial communities differs on petals and leaves. Plant Biol 13:918–924

Kniskern JM, Traw MB, Bergelson J (2007) Salicylic acid and jasmonic acid signaling defense pathways reduce natural bacterial diversity on *Arabidopsis thaliana*. Mol Plant Microbe Interact 20:1512–1522

Kouchi, Imaizumi-Anraku H, Hayashi M, Hakoyama T, Nakagawa T, Umehara Y, Suganuma N, Kawaguchi M (2010) How many peas in a pod? Legume genes responsible for mutualistic symbioses underground. Plant Cell Physiol 51(9):1381–1397

Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69:1875–1883

Millet YA, Danna CH, Clay NK, Songnuan W, Simon MD et al (2010) Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. Plant Cell 22:973–990

Morris CE, Monier J-M (2003) The ecological significance of biofilm formation by plant-associated bacteria. Annu Rev Phytopathol 41:429–453

Newton J, Fray R (2004) Integration of environmental and host derived signals with quorum sensing during plant–microbe interactions. Cell Microbiol 6:213–224
Oldroyd GE (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat Rev Microbiol 11:252–263
Oldroyd GE, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-rhizobial symbiosis. Annu Rev Genet 45:119–144
Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4:701–712
Paungfoo-Lonhienne C, Rentsch D, Robatzek S, Webb RI, Sagulenko E et al (2010) Turning the table: plants consume microbes as a source of nutrients. PLoS One 5:e11915
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
Pirozynski K, Malloch D (1975) The origin of land plants: a matter of mycotrophism. Biosystems 6:153–164
Plett JM, Martin F (2015) Reconsidering mutualistic plant–fungal interactions through the lens of effector biology. Curr Opin Plant Biol 26:45–50
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 (PGPR) and medicinal plants. Springer International Publishing, Switzerland, pp 247–260
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 International Publishing AG, Cham, pp 1–7
Pumplin N, Harrison MJ (2009) Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. Plant Physiol 151:809–819
Rasmussen TB, Givskov M (2006) Quorum sensing inhibitors: a bargain of effects. Microbiology 152:895–904
Roche P, Debellé F, Maillet F, Lerouge P, Faucher C et al (1991) Molecular basis of symbiotic host specificity in *Rhizobium meliloti*: nodH and nodPQ genes encode the sulfation of lipo-oligosaccharide signals. Cell 67:1131–1143
Rodriguez R, White J Jr, Arnold AE, Redman R (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330
Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837
Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H (2013) The biology of strigolactones. Trends Plant Sci 18:72–83
Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y et al (1996) Systemic acquired resistance. Plant Cell 8:1809
Sasaki T, Suzaki T, Soyano T, Kojima M, Sakakibara H, Kawaguchi M (2014) Shoot-derived cytokinins systemically regulate root nodulation. Nat Commun 5:4983. https://doi.org/10.1038/ncomms5983
Schenk ST, Stein E, Kogel K-H, Schikora A (2012) Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal Behav 7:178–181
Scheres B, Van de Wiel C, Zalensky A, Horvath B, Spaink H, Van Eck H, Zwartkruis F, Wolters AM, Gloudemans T, Van Kammen A, Bisseling T (1990) The ENOD12 gene product is involved in the infection process during the pea-*Rhizobium* interaction. Cell 60:281–294
Schulz B (2006) Mutualistic interactions with fungal root endophytes. In: Microbial root endophytes. Springer, Berlin, pp 261–279
Smith D, Zhou X (2014) Preface. Can J Plant Sci 94:995–1008. https://doi.org/10.4141/cjps-2014-503
Spaink HP (2000) Root nodulation and infection factors produced by rhizobial bacteria. Annu Rev Microbiol 54:257–288

Subramoni S, Gonzalez JF, Johnson A, Péchy-Tarr M, Rochat L et al (2011) Bacterial subfamily of LuxR regulators that respond to plant compounds. Appl Environ Microbiol 77:4579–4588

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

Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant Microbe Interact 13:637–648

Thomas P, Sekhar AC (2014) Live cell imaging reveals extensive intracellular cytoplasmic colonization of banana by normally non-cultivable endophytic bacteria. AoB Plants 6:plu002

Trivedi P, Pandey A (2008) Plant growth promotion abilities and formulation of *Bacillus megaterium* strain B 388 (MTCC6521) isolated from a temperate Himalayan location. Indian J Microbiol 48:342–347

van der Heijden MG, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

Velázquez E, Rojas M, Lorite MJ, Rivas R, Zurdo-Piñeiro JL et al (2008) Genetic diversity of endophytic bacteria which could be find in the apoplastic sap of the medullary parenchym of the stem of healthy sugarcane plants. J Basic Microbiol 48:118–124

Venturi V, Fuqua C (2013) Chemical signaling between plants and plant-pathogenic bacteria. Annu Rev Phytopathol 51:17–37

Venturi V, Keel C (2016) Signaling in the rhizosphere. Trends Plant Sci 21:187–198

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13:87–115

Whipps JM (1990) Carbon economy. In: Lynch JM (ed) The rhizosphere. Wiley, Chichester, pp 59–97

Yehuda Z, Shenker M, Hadar Y, Chen Y (2000) Remedy of chlorosis induced by iron deficiency in plants with the fungal siderophore rhizoferrin. J Plant Nutr 23:1991–2006

Zambell CB, White JF (2015) In the forest vine *Smilax rotundifolia*, fungal epiphytes show site-wide spatial correlation, while endophytes show evidence of niche partitioning. Fungal Divers 75:279–297

Zamioudis C, Pieterse CM (2012) Modulation of host immunity by beneficial microbes. Mol Plant Microbe Interact 25:139–150

Chapter 25
Mycorrhizae: A Sustainable Industry for Plant and Soil Environment

Rouf Ahmad Bhat, Moonisa Aslam Dervash, Mohammad Aneesul Mehmood, Bhat Mohd Skinder, Asmat Rashid, Javeed Iqbal Ahmad Bhat, Dig Vijay Singh, and Rafiq Lone

Abstract The rhizosphere is an environment of plant roots in which most of the microbial activities of soil occur. The two vital components of soil rhizosphere are root exudates and soil microbes. Root exudates are the chemical compounds that are secreted by roots and act as a source of food for soil microbes especially for mycorrhizae. These chemical compounds plays significant role in soil microbe and plant interaction. The soil mycorrhizae are important for plant growth development and health. They are the main components that enrich the soil nutrients and maintain the soil health in sustainable manner. Furthermore, they enhance the plant growth regulators, provide defense mechanism to the plants, regulate enzymatic activities, increase rate of photosynthesis and supports in bioremediations, thus acting as eco-facilitator in sustainable agriculture both in terms of production and environmental protection.

25.1 Introduction

The rhizosphere is a soil environment that surrounds the plant roots and is crucial for plant life. This region around the plant root is highly active and is also described as a zone of maximum microbial activity. The microbial population present in this environment is relatively different from that of its surroundings due to the presence of root exudates that serve as a source of nutrition for microbial growth (Burdman et al. 2000). The microorganisms may be present in the rhizosphere, rhizoplane,

R.A. Bhat • M.A. Dervash • M.A. Mehmood • A. Rashid • J.I.A. Bhat • D.V. Singh
Division of Environmental Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Kashmir 190025, India

B.M. Skinder
Department of Environmental Sciences, University of Kashmir, Hazratbal, Srinagar 190006, India

R. Lone (✉)
Department of Botany, SBBS University, Khiala, Jalandhar, Punjab 144030, India
e-mail: rafiqlone@gmail.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_25

root tissue and/or in a specialized root structure called a nodule. Very important and significant interactions were reported among plant, soil, and microorganisms present in the soil environment (Antoun and Prevost 2005). These interactions may be beneficial, harmful and or neutral, and can significantly influence plant growth and development (Adesemoye and Kloepper 2009; Ahmad et al. 2011; Lau and Lennon 2011: Khanday et al. 2016). The microorganisms colonizing plant roots generally include bacteria, algae, fungi, protozoa and actinomycetes. Enhancement of plant growth and development by application of these microbial populations is well evident (Bhattacharyya and Jha 2012; Gray and Smith 2005; Hayat et al. 2010; Saharan and Nehra 2011). Fungi represent a significant portion of soil rhizosphere microflora and influence plant growth. The symbiotic association generated by fungi with plant roots (mycorrhizae) increases the root surface area, and therefore enables the plant to absorb water and nutrients more efficiently from large soil volume. The mycorrhizal association not only increases the nutrient and water availability, but also protects the plant from a variety of abiotic stresses (Evelin et al. 2009; Miransari 2010). Mycorrhizae play significant role in enhancing plant growth by way of different mechanisms. Exploring the mechanisms of growth promotion by mycorrhizae could be very useful for enhancing plant growth by using these microbial populations together, particularly under stressful environments. Although a number of studies have shown that combined application of mycorrhizae could be a meaningful approach for sustainable agriculture (Denton 2007; Najafi et al. 2012; Ordookhani et al. 2010), there are still certain aspects which need further investigations for obtaining maximum benefits in terms of improved plant growth from this naturally occurring population. The give and take, and vice-versa association between plant roots and fungi has great impact on crop quantity and quality. Ectomycorrhizae and arbuscular mycorrhizae are the two common kinds of fungi involved in such interactions and certainly the most plentiful fungi that are normally present in agricultural soils. They form symbiotic association with terrestrial as well as aquatic plants (Christie et al. 2004; Liu and Chen 2007; Willis et al. 2013). Almost 75–80% of all plants, including, terrestrial, aquatic mostly agricultural, horticultural, and hardwood crop species are able to establish this kind of symbiotic interaction that benefits both plant and fungi. These fungi enter into root cortical cells and form a particular haustoria-like structure called arbuscule that acts as a mediator for the exchange of metabolites between fungus and host cytoplasm (Oueslati 2003). The mycorrhiza hyphae also proliferate into the soil which helps plants to obtain mineral nutrients and water from the soil and also contribute to improving soil structure (Javaid 2009; Rillig and Mummey 2006). AM fungi play a very important role in ecosystems through nutrient cycling (Shokri and Maadi 2009; Wu et al. 2011a, b; Yaseen et al. 2012). Growth and productivity of several field crops have been observed by root colonization of mycorrhizal fungi (Cavagnaro et al. 2006; Nunes et al. 2010). Mycorrhizae increase the availability and supply of slowly diffusing ions, such as phosphate to the plant (Sharda and Koide 2010). In addition to their significant role in P acquisition, AM fungi can also provide other macro- and micro-nutrients such as N, K, Mg, Cu and

Zn, particularly in soils where they are present in less soluble forms (Meding and Zasoski 2008; Smith and Read 2008).

25.2 Mycorrhizae to Improve Plant Growth Under Extreme Adverse Conditions

The growth support due to mycorrhizal relationship can be described by numerous ways of mechanisms performed by fungi under different conditions. These include release of metabolites viz., phytohormones, amino acids, vitamins, mineralization and solubilization processes. In addition to providing nutritional and structural benefits to plants, they also impart other benefits to them including production/accumulation of secondary metabolites, osmotic adjustment under osmotic stress, improved nitrogen fixation, enhanced photosynthesis rate, and increased resistance against biotic and abiotic stresses (Khaosaad et al. 2007; Ruiz-Lozano 2003; Schliemann et al. 2008; Selvakumar and Thamizhiniyan 2011; Sheng et al. 2009; Shinde et al. 2013; Takeda et al. 2007; Wu and Xia 2006). Many researchers have reported that mycorrhizae fungi can improve plant tolerance to heavy metals, drought, and salinity, and also protect plants from pathogens (Azcon-Aguilar et al. 2002; Gamalero et al. 2009a, b; Gosling et al. 2006; Marulanda et al. 2006, 2009; Vivas et al. 2003; Zhang et al. 2010). They can also improve crop growth and yield by alleviating the negative influence of allelochemicals (Bajwa et al. 2003; Khanday et al. 2016). These effects can be described by number of different ways of mechanisms that may differ depending on plant and mycorrhizae association as well as internal and external stress factors. For example, a number of studies have shown that improved P nutrition under salinity and water deficit environment is a primary mechanism for promoting stress tolerance in plants (Cantrell and Linderman 2001; Colla et al. 2008; Feng et al. 2002). There are many reports which show that mycorrhizae fungi can increase soil enzyme activities, such as phosphatase (Mar Vazquez et al. 2000). Some studies have also demonstrated that mycorrhizae association not only influences P nutrition but also affects the physiological processes of plants by increasing proline contents (Ruiz-Lozano et al. 1995). Proline is known to act as an osmoregulator under stress conditions (Ashraf and Foolad 2007). The mechanisms used by mycorrhizae to alleviate effects induced adverse conditions of salinity on plant growth include: improvement of plant nutrition, variation in Na^+ and K^+ uptake, modification in physiological and enzymatic activities and alteration of the root architecture to facilitate water uptake (Evelin et al. 2009; Gamalero et al. 2010; Zhang et al. 2011; Zolfaghari et al. 2013). Physiological processes involved in osmoregulation like enhanced CO_2 rate, water use efficiency, and stomatal conductance are also influenced by the activities of mycorrhizae fungi (Birhane et al. 2012; Ruiz-Lozano and Aroca 2010). Mycorrhizae also increase the nitrogen availability of host plant under drought conditions (Subramanian and Charest 1999). It has been shown that mycorrhizal plants absorb

water more efficiently under water deficit environment (Khalvati et al. 2005) that might be due to modification in root architecture which results in better root growth due to numerous branched roots (Berta et al. 2005). As abscisic acid regulates the stomatal conductance by closing stomata under water limited environment, the positive effect of mycorrhizae fungi on plant growth and development under drought stress might be due to its influence on abscisic acid concentration in plants (Jahromi et al. 2008). It has also been observed that mycorrhizae fungi increase salinity tolerance of host plants by improving water status of the inoculated plants by facilitating water transport in plants (Ouziad et al. 2006). Mycorrhizae also enhance soluble sugars and electrolyte concentrations in host plants. For example, improved osmoregulation capacity in mycorrhizae inoculated maize was related to higher soluble sugar and electrolyte concentrations (Feng et al. 2002). Porcel and Ruiz-Lozano (2004) and Al-Garni (2006) also reported increased sugar concentrations in mycorrhizal plants of soybean and *Phragmites australis*. It is also well documented that mycorrhizae fungi affect the expression of a number of antioxidant enzymes (Gamalero et al. 2009a, b), which protect the plants from reactive oxygen species produced under stress conditions. Similarly, improved nodulation due to increased activities of these enzymes under salinity stress has been observed along with other factors such as leghemoglobin content, nitrogenase activity and polyamine contents (Gamalero et al. 2009a, b; Garg and Manchanda 2008; Matamoros et al. 2010; Sannazzaro et al. 2007; Yaseen et al. 2012). Another mechanism used by mycorrhizae fungi to facilitate plant growth under salinity stress is the regulation of plant nutrition. High Na^+ concentration under salinity stress is detrimental for normal plant growth and low K^+/Na^+ ratio has been observed generally in salt sensitive plants (Ashraf et al. 2004). Therefore, improved K^+/Na^+ ratio is believed to be a potential indicator of salinity tolerance in most plants. The AM fungi also play an important role in maintaining a high K^+/Na^+ ratio in host plants exposed to saline conditions (Giri et al. 2007; Sannazzaro et al. 2006; Selvakumar and Thamizhiniyan 2011; Zhang et al. 2011). In general, mycorrhizae enhance plant growth under stressful environments by a number of mechanisms such as regulation of plant nutrition, production of hormones and antioxidant enzymes, and regulation of a multitude of physiological processes. However, it is also evident from the above discussion that the effectiveness of these mechanisms also depends on the extent of mycorrhizae and host plant association as well as a number of soil and plant factors.

25.3 Phytohormones

Plant growth regulators levels have been observed to change during mycorrhizae fungi development reported by many researchers and almost all hormones have been proposed as important regulators of the symbiosis (Hause et al. 2007; Ludwig-Muller 2010; Foo et al. 2013). Moreover, many of these hormones have been shown to be involved in root morphogenesis that enhances the development and

improvement growth of plants especially crops (Rouached et al. 2010; Chiou and Lin 2011; Hammond and White 2011; Sato and Miura 2011; Niu et al. 2013).

25.3.1 Auxin

Auxin is an essential regulator of plant growth and developmental processes. In the roots, it positively regulates the size of the root apical meristem by promoting cell division antagonistically to cytokinin, and it is involved in the regulation of cell elongation with ethylene (Muday et al. 2012; Vanstraelen and Benková 2012). Moreover, it is the main regulator of each lateral root (LR) formation step (Fukaki and Tasaka 2009; De Smet 2011). Elevated levels of auxin, either due to exogenous application or to enhanced biosynthesis, are sufficient to increase lateral root formation, while mutations that reduce auxin signalling, such as solitary root of Arabidopsis, cause a strong reduction in lateral root formation (revised by Ivanchenko et al. 2008). Since mycorrhizae colonization increases root branching, the involvement of auxin in the root system architecture regulation of mycorrhizal plants has been suggested (Ludwig-Muller 2010; Hanlon and Coenen 2011; Sukumar et al. 2013). Auxin is involved in the mycorrhizae host–fungus interaction. The addition of auxin has been shown to increase spore germination and hyphal growth, and to influence the infection rate and percentage of colonization (Ludwig-Muller 2010). The auxin level in plant tissues increases in different plant–fungus associations (Ludwig-Muller 2010), probably independently of fungus production (Jentschel et al. 2007; Ludwig-Muller 2010).

25.3.2 Cytokinins

Cytokinins play a crucial role in regulating the proliferation and differentiation of plant cells, and also control many developmental processes. They are recognized as essential regulators of the plant root system, as they are involved, antagonistically to auxin, in the control of the size of the root apical meristem, and in the rate of root growth and lateral roots organogenesis (Sakakibara 2006; Werner et al. 2010; Marhavý et al. 2011). They can redirect assimilates and induce invertases, thus contributing directly to the plant carbon redistribution (Ludwig-Muller 2010). However, recent studies have suggested that cytokinins might not be involved to any great extent in the regulation of mycorrhizal development (Foo et al. 2013). A number of mycorrhizae plants accumulate more cytokinins than non mycorrhizal plants in both the shoots and the roots (Torelli et al. 2000; Shaul-Keinan et al. 2002). Since the main sites of cytokinin synthesis include the root tips (Aloni et al. 2006), the high cytokinin level found may be, in part, a consequence of increased root branching.

25.3.3 Ethylene

Ethylene plays an important role in coordinating internal and external signals, as well as in several stress responses and interaction of plants with other organisms (Lei et al. 2010; López-Ráez et al. 2010). In mycorrhizae symbiosis, ethylene and salicylic acid function as negative regulators of mycorrhizal intensity (Gamalero et al. 2008; Ludwig-Muller 2010). In fact, a strong ethylene inhibitory effect has been observed on early symbiotic gene expression, on fungus entry into roots (Mukherjee and Ané 2011) and on intra radical fungal spread (Martín-Rodríguez et al. 2011). The ethylene content is increased by a deficiency of ABA, which is in contrast necessary for arbuscule formation and is positively correlated to mycorrhizal establishment (Ludwig-Muller 2010; Martín-Rodríguez et al. 2011). Accordingly, most researchers indicate that ethylene production is diminished in mycorrhizae-infected plants (López-Ráez et al. 2010), although a few contradictory results have also been reported (Dugassa et al. 1996). Ethylene, like auxin and cytokinin, is an important regulator of root morphogenesis. It inhibits root elongation by reducing cell elongation synergistically with auxin (Muday et al. 2012). However, it also acts antagonistically to auxin by inhibiting LR formation in the earliest stages of LR initiation, as has been shown through treatments with ethylene.

25.4 Mycorrhizae Symbiosis a Sustainable Ecosystem Services

Plants in ecosystems perform a series of functions that are beneficial to the well-being of humans, providing multiple resources and processes (Daily 1997). Trade-offs and links between plants and soil microbial communities can act as drivers of a wide range of processes in ecosystems (Lavorel 2013; Grigulis et al. 2013). Given the beneficial functions of mycorrhizae fungi on plant fitness, resilience against environmental stresses, nutrient cycling, and soil quality, mycorrhizae symbiosis is now recognized to play a fundamental role as a provider of ecosystem services. Various ecosystem services delivered by mycorrhizae have been identified: biofertilization from the mycorrhizae promotion of plant growth, which in turn reduces fertilizer requirements, stabilization of soil structure, and bioregulation consequent to the plant metabolic modifications by mycorrhizae fungi (Gianinazzi et al. 2010). Linking functional traits of plants and soil microbes, such as mycorrhizae fungi, with their delivery of multiple ecosystem services is currently considered a rational mean for assessing the functioning of a given ecosystem (De-Bello et al. 2010). Less attention, however, has been given to beneficial soil organisms in general and mycorrhizae in particular and their influence on the processes of ecosystems that contribute to the ecosystem services in agroecology. The positive effect of mycorrhizae on the ability of plants to counteract the conditions of drought confers to mycorrhizae a pivotal role as a valuable technology

not only for the sustainability of agricultural systems, but also for the restoration of degraded natural arid and semi-arid areas, where multiple environmental stresses, including drought, occur (Gianinazzi et al. 2010; Barea et al. 2011). In light of the assessment of the multiple ecosystem services provided by mycorrhizae, critical advances are required for elucidating the functional importance and value of plant and mycorrhizal diversity that are necessary for the functioning of ecosystems. These are also required for clarify the links among plant traits and their associated mycorrhizae fungal characteristics to quantify the contribution of plant–mycorrhizae fungi associations to ecosystem services under various environmental constraints (Barea et al. 2011; Grigulis et al. 2013; Lavorel 2013). The role of mycorrhizae symbiosis in the functional traits of both plants and microbes that could characterize above- and belowground ecosystem services has not yet been explored. The application of a trait-based approach to both plant and mycorrhizae fungal communities represents a promising opportunity to understand how functional mycorrhizae feedbacks between plant and mycorrhizae fungi translate into interactions between ecosystem services (Lavorel 2013).

25.5 Mycorrhizal Association Imparting Drought Tolerance in Crop Plants

Mycorrhizal association improving drought tolerance of agronomically important crop plants and has been reported by earlier workers in crops like wheat, soybean, onion, capsicum, maize, barley, cotton, etc. (Beltrano et al. 2013; Maya and Matsubara 2013). Improved growth and development of mycorrhizal plants especially in stressful environment is partly attributed to better water status of the leaf tissues (Colla et al. 2008), improved abilities to absorb nutrients from soil, higher root hydraulic conductivity and high photosynthetic rates of mycorrhizal plants (Yang et al. 2014). Therefore, it is evident that the mycorrhizal fungal association offers a number of benefits to the plants. Although the association is costly to the plant as it has to shell down some amount of carbon to the fungi, still the benefits derived in terms of protecting the plants under stress is much more than what the plant is losing. Under the conditions of water deficit, the external mycelium of *R. intraradices* may have a direct role in transport of considerable amount of nitrogen in the form of NO^{-}_{3} as observed in maize plants where roots also had higher glutathione reductase activity and P status in host plants (Subramanian and Charest 1999). In general, drought affects the AMF colonization negatively (Ryan and Ash 1996), in wheat AMF has been shown to alleviate the drought stress and increase yield mainly through improved nutrient uptake (Al-Karaki et al. 2004). *Claroideoglomus claroideum* (*Glomus claroideum*) seems to play a key role in imparting drought tolerance in wheat by improved chlorophyll content and cell membrane permeability (Beltrano and Ronco 2008). Various horticultural crops have been shown to tolerate drought via AMF. For example, Wu et al. (2013) have

reported that the AMF inoculated citrus exhibited higher drought tolerance than the non-AMF citrus. In lettuce, an important vegetable crop in Europe, the AMF association promoted secondary metabolite production thereby making the plants to withstand abiotic stress (Baslam and Goicoechea 2012). AMF has been shown to be associated with date palms indicating possible role of the AMF in withstanding droughts of deserts (Symanczik et al. 2014).

Inoculation of tomato plants with *R. intraradices* resulted in improved nutritional status, increased shoot dry matter, fruits and flowers with higher quantities of ascorbic acid and total soluble solids (Subramanian et al. 2006). Ballesteros-Almanza et al. (2010) observed that inoculation of common bean with AMF imparted drought tolerance by improving intraradical and extraradical hyphae, arbuscule development, and succinate dehydrogenase and alkaline phosphatase activity in root system. The list of AMF conferring drought tolerance on different crop species is given in Table 25.1.

25.6 Arbuscular Mycorrhizal Fungi as a Tool to Improve the Phytoremediation

Many large areas around the world are contaminated with heavy metals and organic compounds; most of these have not been remediated due to the high cost and technical drawbacks of currently available technologies. Heavy metals tend to accumulate in soils and aquatic sediments and can enter the food chain leading to the biomagnification phenomenon thereby representing a risk to the environment and to human health (Clijsters et al. 1999). Some essential elements, such as copper (Cu) and zinc (Zn), may be present in soils and waters at potentially toxic levels mainly as a result of agricultural and industrial practices (Ali et al. 2004). Alternative techniques for the clean-up of polluted soil and water, such as the cost-effective and less disruptive phytoremediation, have gained acceptance in recent years (Pilon-Smits 2005; Thewys et al. 2010). Trees have been suggested as suitable for phytoremediation due to their high biomass production (Dickinson and Pulford 2005) and because tree plantations can be multi-purpose (Tognetti et al. 2013). Poplar has many characteristics suitable for phytoremediation: a fast rate of growth, a deep and wide-spreading root system and a metal-resistance trait (Aronsson and Perttu 2001; Di Baccio et al. 2011; Sebastiani et al. 2004). Plant symbiotic fungi, such as mycorrhizae, and soil bacteria can confer increased tolerance to stress (Gamalero et al. 2009a, b). Arbuscular mycorrhizal fungi (AMF) form associations with the roots of the vast majority of land plants; the fungus colonizes the roots and forms arbuscules within root cortical cells thus improving plant nutrient uptake, especially phosphorus (Smith and Read 1997a, b; Khanday et al. 2016). Moreover, increasing evidence shows that symbiotic fungi contribute to plant adaptation to multiple biotic and abiotic stresses (Gohre and Paszkowski 2006; Lebeau et al. 2008, Lingua et al. 2002; Liu et al. 2007; Rodriguez and Redman 2008; Smith et al.

Table 25.1 Examples of AMF conferring drought tolerance in crop plants

Species	Crop	Stress	Mechanism	Reference
Claroideoglomus etunicatum	Maize	High temperature	Reduced membrane lipid peroxidation, membrane permeability and increased accumulation of osmotic adjustment compounds and antioxidant activity	Zhu et al. (2010)
Glomus versiforme	Citrus (*Citrus tangerine*)	Drought	Higher activities of catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD)	Wu et al. (2006)
Funneliformis mosseae	Maize (*Zea mays*)	Drought	Accumulation of amino acids and imino acids, remarkable increase in trehalose content and higher trehalase activity	Schellenbaum (1998)
Funneliformis mosseae and *Claroideoglomus etunicatum*	Wheat (*Triticum asetivum*)	Drought	Higher biomass and higher grain yields, shoot P and Fe concentration in mycorrhizal plants	Al-karaki et al. (2004)
Glomus spp.	Wheat (*Triticum asetivum*)	Water stress	Mycorrhiza increased the content of free amino acids, proline, total soluble and crude protein, total carbohydrate, total soluble and insoluble sugars, and enhanced the activity of antioxidant enzymes like peroxidase (POX) and catalase (CAT)	Khalafallah and Abo-Ghalia (2008)
Rhizophagus intraradices	Sorghum (*Sorghum bicolor*)	Drought	Mycorrhiza minimized the adverse effect of drought and increased the grain yield by 17.8%	Alizadeh et al. (2011)
	Soybean (*Glycine max*)	Drought	Higher leaf water potential in mycorrhizal plants, and mycorrhiza protected the plants against oxidative stress	Porcel and Ruiz-Lozano (2004), Meddich et al. (2015)

(continued)

Table 25.1 (continued)

Species	Crop	Stress	Mechanism	Reference
Funneliformis mosseae Rhizophagus diaphanum Glomus versiforme	Trifoliate orange (*Poncirus trifoliata*)	Drought	Higher plant growth and biomass, acid and total phosphatase activity, leaf and root P contents in drought stressed mycorrhizal seedlings particularly in *F. mosseae*	Wu et al. (2011a, b)
Funneliformis mosseae	Sunflower	Drought	Inoculated plants produced more dry matter, heavier seeds and greater seed and oil yields with *F. mosseae*. Despite of reduction in N percentage due to drought, N percentage was higher in inoculated plants compared to control	Gholamhoseini et al. (2013)
Glomus spp.	*Boswellia papyrifera*	Pulsed water availability conditions	Higher level of AM colonization under irregular precipitation regime where mycorrhizal seedlings had higher biomass, increased transpiration, higher water	Birhane et al. (2012)
Funneliformis mosseae + *Rhizophagus intraradices*	Lettuce (*Lactuca sativa*.)	Water deficit	Under water deficit, the accumulation of potential antioxidants (mainly carotenoids, anthocyanins and to a lesser extent chlorophyll and phenolics) in the leaves of mycorrhizal lettuce plants were more. Shoot biomass in AM lettuce under moderate water deficit was equal to well watered AM plants. Improved lettuce quality and reduced irrigation without affecting lettuce production	Baslam and Goicoechea (2012)

2010). In the case of heavy metals, the beneficial effect varies according to plant and fungal species, metal and concentration (Bois et al. 2005; Lebeau et al. 2008; Takacs et al. 2005; Todeschini et al. 2007). The mechanisms by which AMF offer protection from stress has not been clarified, although decreased metal uptake has been reported in some cases (Christophersen et al. 2012; Mrnka et al. 2012). The potential of plant–microbe interactions in enhancing phytoremediation potential has been reviewed extensively elsewhere (Doty 2008; Lebeau et al. 2008; Rajkumar et al. 2012; Khanday et al. 2016). Also in poplar, the effects of bacterial endophytes (van der Lelie et al. 2009), and of endo- and ectomycorrhiza (Mrnka et al. 2012) on phytoremediation capacity have been described. Information regarding basic molecular processes underlying metal detoxification/tolerance is scarce especially in tree species. Metallothioneins are among the plant components that respond to metal stress. They are small proteins encoded by a multigene family whose members appear to be differentially regulated in relation to organ and developmental stage, and in response to a number of stimuli including heavy metals (Cobbett and Goldsbrough 2002). A role for metallothioneins in heavy metals detoxification and homeostasis has been proposed either because they bind to HMs or because they function as antioxidants (Akashi et al. 2004). The evidence is largely based on metallothioneins gene expression studies and yeast complementation experiments with plant metallothionein genes, and some of it comes from studies on poplar species or hybrids (Balestrazzi et al. 2009; Castiglione et al. 2007; Hassinen et al. 2009; Kohler et al. 2004).

25.7 Significance of Arbuscular Mycorrhizal Fungi (AMF) in Global Sustainable Development

25.7.1 Soil Fertility

Arbuscular mycorrhizal fungi can overcome nutrient limitation to plant growth by enhancing nutrient acquisition (Clark and Zeto 2000). Most studies have investigated P uptake but mycorrhizae have been implicated in the uptake of other essential nutrients also (Khanday et al. 2016). The increase in inorganic nutrient uptake in mycorrhizal plants is mainly because fungal hyphae provide the large surface area for nutrient acquisition to external root surface as compared to uninfected roots. As the fungal mycelium grows through soil, it scavenges for mineral nutrients and is able to make contact with uninfected roots, sometimes of different host species.

25.7.2 Phosphorus Uptake

Phosphorus is a major plant nutrient required in relatively large amounts and plays a vital role in all biological functions in energy transfer through the formation of energy-rich phosphate esters and is also an essential component of macromolecules such as nucleotiodes, phospholipids and sugar phosphates (Marschner 1995). The benefits of mycorrhizae are the increase in the phosphorus uptake by the plant. The general process of phosphorus uptake consists of three sub-processes; (i) absorption from soil by AMF hyphae, (ii) translocation along the hyphae from external to internal (root cortex) mycelia, (iii) the transfer of phosphate to cortical root cells (Barea 1991). The various mechanisms proposed to account for enhanced nutrient uptake include (i) increased exploration of soil; (ii) increased translocation of phosphorus into plants through arbuscules; (iii) modification of root environment; (iv) efficient utilization of P within plants; (v) efficient transfer of P to plant roots; and (vi) increased storage of absorbed P. Uptake of phosphate by roots is much faster than diffusion of ions to the absorption surfaces of the root (Bhat and Kaveriappa 2007). This causes phosphate depletion zone around the roots. The extensive extrametrical hyphae of AMF extend out into the soil for several centimeters so that it bridges the zone of nutrient depletion.

25.7.3 Nitrogen Uptake

Nitrogen (N) is essential for the formation of amino acids and is indirectly involved in protein and nucleic acid synthesis. AMF associated plants have increased N content in shoots. A number of mechanisms are suggested for this effects, namely (i) improvement of symbiotic nitrogen fixation; (ii) direct uptake of combined nitrogen by mycorrhizal fungi; (iii) facilitated nitrogen transfer, a process by which a part of nitrogen fixed by nodulated plants benefits the non-nodulated plants; (iv) increased enzymatic activities involved in nitrogen metabolism like pectinase, xyloglucanase and cellulose which are able to decompose soil organic matter (Barea 1991). The hyphae of AMF have the tendency to extract nitrogen and transport it from the soil to plants. They contain enzymes that breakdown organic nitrogen and contain nitrogen reductase which alters the forms of nitrogen in the soil. AM improves growth, nodulation and nitrogen fixation in legume-*Rhizobium* symbiosis. According to McFarland et al. (2010) more than 50% of plant N requirement is supplied by mycorrhizal association. Mycorrhizal inoculation enhanced activities of nitrate reductase, glutamine synthetase and glutamine synthase in the roots and shoots of mycorrhizal corn (*Zea mays* L.) as reported by Subramanian and Charest (1999). Recently, a plant ammonium transporter, which is activated in the presence of AMF has been identified and indicated that the way by which N is transferred in plant may be similar to P transfer (Guether et al. 2009).

25.7.4 Supply of Organic Mineral Nutrients

Although many mycorrhizal fungi can access inorganic forms of N and P, some litter-inhabiting mycorrhizal fungi produce proteases and distribute soluble amino compounds through hyphal networks into the root and *Glomus* has been shown to transport the amino acids glycine and glutamine into wheat (Hawkins et al. 2000).

25.7.5 Micronutrients

The extrametrical hyphae of AMF take up and transport potassium (K), calcium and sulphates and AM colonization affects the concentration and amounts of K in shoots. AM plants accumulate large quantities of some micronutrients (Zn, Cu, Co) under conditions of low soil nutrient availability (Feber et al. 1990). The absorption is attributed to the uptake and transport by external hyphae due to wider exploration of soil volume by extended extrametrical hyphae. Uptake and concentration of manganese (Mn) in plants may not be affected by AM and more often it may be lower in AM plants, thus contributing to higher Mn tolerance in plants. The enhanced iron (Fe) uptake may be due to specific Fe chelators.

25.7.6 Water Uptake in Mycorrhizal Association

AMF also play an important role in the water economy. The AMF association improves the hydraulic conductivity of the roots and improves water uptake by the plants or otherwise alters the plant physiology to reduce the stress response to soil drought (Safir and Nelson 1985). Mycorrhizal plants show better survival than non-mycorrhizal plants in extreme dry conditions. It reveals that mycelial network extends deeper and wider in the soil in search of water and nutrients. The permeability of cell membrane to water may also be altered by mycorrhizal colonization though the improved phosphorus nutrition and colonization by AMF can improve the drought resistance of plants (Sylvia and Williams 1992). Under conditions of drought stress, AMF exert their influence by increasing the transpiration rate and lowering stomatal resistance or by altering the balance of plant hormones (Huang et al. 1985). The change in leaf elasticity due to AMF inoculation improves water and turgor potential of leaf and also increase root length and depth (Kothari et al. 1990) and may also influence water relations and therefore, the drought resistance of the plants.

25.7.7 Soil Aggregation and Soil Stabilization

Disturbances in ecosystem affect the physical, chemical and biological processes in the soil. AMF help in the binding of soil particles and improve soil aggregation and soil conservation (Dodd 2000). Arbuscular mycorrhizal fungi are also known to enhance soil fertility, as they produce glomalin which upon accumulation in soil, along with the AMF hyphae forms micro aggregates and finally macro aggregates and, thus, acts as a backbone for soil aggregation and soil stabilization directly. It also releases exudates in the soil and thus promotes aggregate stability and also boost up other microorganism growth (Johnson et al. 2002; Khanday et al. 2016).

25.7.8 Role of AMF in Wasteland Reclamation

AMF have a great potential in the recovery of disturbed lands and these can be used in reclamation of wastelands. Inoculation with AM fungi can improve the growth and survival of desirable revegetation species. Colonization with AMF can cause a beneficial physiological effect on host plant in increasing uptake of soil phosphorus (Gerdemann 1975). Nicolson (1967) suggested that plant growth in wastelands could be effectively improved by incorporating AMF. It has been suggested that many plants may require mycorrhizal infection in order to survive on disturbed land. The absorptive surface area contributed by soil mycelium allows phosphorus uptake from a much greater volume. Host growth is also enhanced particularly in phosphorus-deficient soils (Mosse 1973). AM fungi have been conclusively shown to improve revegetation of coal spoils, strip mines, waste areas, road sites and other disturbed areas (Jha et al. 1994). Addition of AMF provides a nutritional advantage to associated plants in addition to providing possible resistance to low pH, heavy metal toxicants and high temperature. Presence and utilization of AMF has markedly increased the success of rehabilitation to these moisture deficient zones. Pre-inoculation of nursery seedlings with appropriate mycorrhizal fungi would benefit in revegetation of disturbed mined land. Rani et al. (2001) from our laboratory had worked on the effect of *Glomus mosseae, G. fasciculatum* along with *Rhizobium* and *Trichoderma* on better biomass yield of *Prosopis cineraria* and *Acacia nilotica* and reported further that co-inoculation with AM and *Rhizobium* resulted in maximum growth and best nodulation.

25.7.9 Role of AMF in Agriculture

The AMF symbiosis has also been shown to contribute substantially to soil conservation via its role in the formation of water-stable soil aggregates by the extrametrical hyphae. These aggregates are crucial for creating and maintaining a

macroporous, water permeable soil structure, which is prerequisite for erosion resistance and also necessary for efficient nutrient cycling. The profuse use of phosphate fertilizers and chemicals causes pollution problems and health hazards. So the use of AMF is being encouraged in agriculture. The exploitation of mycorrhizal fungi is not easy because large scale production of AMF on field scale is not yet possible. But there is a possibility of mass production of AMF by means of appropriate crop and soil management practices. More farm management practices can influence the types of AMF found in agriculture soils. Apart from effects of fertilizer application on AMF, other practices like crop rotation, minimal cultivation, monoculture, tillage, organic amendments, and application of biocides affects the AMF (Kaur and Mukerji 1999) Mycorrhizal symbiosis plays an important role in the tropical agricultural crops because in tropical region, the soil is phosphorus deficient. Mosse (1973) reported that 75% of the phosphorus applied to the crops is not utilized by them but get converted to forms unavailable to plants.

25.7.10 Crop Dependency on Mycorrhizae

The relative dependency on AMF for nutrient uptake in crop plants depend on root factors such as surface area, root hair abundance and length, growth rate, response to soil conditions and exudations (Smith and Read 1997a, b). Crops such as corn (*Zea mays*) and flax (*Linum usitatissiumum*) are highly dependent on AMF to meet their early phosphorus requirements. Legumes, beans and potatoes also benefit significantly from mycorrhizae. Barley, wheat and oat benefit from mycorrhizal symbiosis.

25.7.11 Crop Rotation

It has been well established that the AMF activity is decreased by non mycorrhizal fungi host plants and highly mycorrhizal host crop increase AMF inoculum potential of the soil and colonization of the subsequent crops (Karasawa et al. 2002). An increase in AMF colonization and growth in maize occurred following sunflower (*Helianthus annuus,* mycorrhizal) when compared to corn following mustard (non-mycorrhizal). Here non mycorrhizal plants in the rotation reduce the rate of AMF colonization in following crops. Gavito and Miller (1998) also observed delayed AMF colonization of corn (*Zea mays*) following canola (*Brassica napus*); a non-mycorrhizal host species, when compared to the colonization of corn following the AMF host species bromegrass (*Bromus* spp.) and alfalfa (*Medicago sativa*).

25.7.12 Phosphorus Fertility

The benefits of AMF are greatest in systems where P in the soil is low. As the level of P available to plants increases, the plant tissue phosphorus also increases and the plant carbon investment in mycorrhizae is not economically beneficial to the plant (Grant et al. 2001). Encouragement of mycorrhizal symbiosis may increase early uptake of phosphorus, improving crop yield potential without starter P fertilizer application (Grant et al. 2005).

25.7.13 Seedling Establishment

AMF also play an important role in successful reforestation and there are several reports of increased establishment of many of forest seedlings in the field, like *Quercus rubra* (Dickie et al. 2001). In a study conducted by Ramos-Zapata et al. (2006) on establishment of *Desmoncus orthacanthos* along with inoculation of AM fungi resulted in a threefold increase in survival of seedlings in the field.

25.7.14 Alleviation of Environmental Stress

AMF are able to alter plant physiological and morphological properties in a way by which plant can handle the stress (Miransari et al. 2008). AM fungi facilitate better survival of plants under stress conditions through a boost up in uptake of nutrients particularly P, Zn, Cu and water. They make the host resilient to adverse conditions created by unfavourable factors related to soil or climate. The role played by these fungi in alleviating the stress on the plant due to drought, metal pollution, salinity and grazing is briefly described.

25.7.15 Water Stress

Water stress is a major agricultural constraint in the semi-arid tropics. It is well known to have a considerable negative impact on nodule function. It inhibits photosynthesis and disturbs the delicate mechanism of oxygen control in nodules. The latter is essential for active nitrogen fixation. AMF symbiosis can protect host plants against detrimental effects caused by water stress. Quilambo (2000) reported that inoculation with indigenous inoculants resulted in increased leaf and root growth and prevented the expected increase in root to shoot ratio and root–weight ratio that is normally observed under phosphorus deficient and water stress conditions in peanut. AMF improve the uptake of nutrients like N and P in water stressed

conditions (Tobar et al. 1994). Water scarcity in soil is conveyed to the shoots by means of non-hydraulic chemical signal that is relayed from the dehydrating roots to the aerial shoots by the transpiration system. The response is expressed by the leaves in terms of stunted growth and decreased stomatal conductance. AMF alters this non-hydraulic root–to–shoot signaling of soil drying by eliminating the leaf response (Auge et al. 1986). The extraradical AMF hyphae increase the absorptive surface area of the roots (Hampp et al. 2000) which in turns reduces the resistance to water uptake. Hence, the role played by AMF in alleviating water stress of plants has been investigated and it appears that drought resistance is enhanced. An increase reliance on AMF for nutrient uptake can frequently be detected. Hence, AMF help to alleviate the water stress conditions.

25.7.16 Increased Resistance Against Root Pathogens

AM are intimately associated with their host plants, particularly the roots. Therefore, an interaction between the symbionts and plant pathogens is bound to occur. By creating new environments in their zone of influence, AMF contribute to the proliferation of specific microorganisms, a few of them interact with pathogens by antibiosis, competition and parasitism (Filion et al. 1999). Plants are subject to attack by various organisms ranging from fungi, bacteria, viruses and nematodes. Mycorrhizal plants usually suffer less damage from infection than non-mycorrhizal plants (Dehne 1982; Filion et al. 1999). Soybean colonized with *Glomus mosseae* grown in soils infested with pathogenic *Macrophomina phaseolina, Fusarium solani* and *Rhizoctonia solani* had growth greater or comparable to plants grown in without AMF inoculated soils. Mycorrhizal tobacco and alfalfa are reported to be resistant to a plethora of fungal pathogens like *Phytophthora megasperma, Pyrenocheata terrestris*, *Fusarium oxysporum, Pythium ultimum* etc. (Kaye et al. 1984; Schenk 1981). Several mechanisms have been proposed to explain the protection extended by AMF to host plants against attack by pathogens. Mycorrhizal root tissues are more lignified than non-mycorrhizal ones, particularly in the vascular region. This restricts the endophyte to the cortex. The same mechanism may hold back the invading organism too (Dehne 1982) increasing root thickenings, and causing chemical differences. Amino acid content, particularly arginine has been found to be high in AM plants. AMF altered physiology of roots may prevent penetration and retard the development of nematodes (Schenk 1981). Some authors have suggested that improved nutrition may protect the plant against pathogens. Mycorrhizal fungi are believed to induce high activation of antimicrobial phenyl propanoid metabolism in roots. It has been reported that induced resistance of AMF sweet orange to *Phytophthora* root–rot disease does not appear to operate unless a P nutritional advantage is conferred on the AMF plant (Graham and Egel 1988).

25.7.17 Carbon Cycling

Significant amount of carbon flows through mycorrhizal mycelia to different components of soils. Production of glycoproteins such as glomalin that are involved in the formation and stability of soil aggregates may have also an important influence on other microorganisms associated with the AMF mycelium (Johansson et al. 2004).

25.7.18 Biohardening Tool

The technique of using AM fungi in micropropagation has been applied recently for clonal selection in woody plants (Salamanca et al. 1992). The inoculation of AMF to nursery plants has been proved both necessary and feasible and it has been extended to micropropagated plants (Adholeya et al. 2005). Salamanca et al. (1992) studied mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified Mediterranean ecosystem. Inoculation of micropropagated plantlets with active culture of AMF appeared to be critical for their survival and growth (Yadav et al. 2011). This avoids 'transient transplant shock' and stunted growth on transfer in the field. Endomycorrhization can modify root architecture to give a root system which is better adapted for uptake of mineral nutrients and water as well as increasing hormone production and resistance to pesticides and root pathogens. Micropropagated plantlets inoculated with AM spores increases the survival rate and growth in potted conditions.

25.7.19 Ornamental Flowering Plants

Vase life is an important consideration of choosing flowers. The longevity or potential of vase life of flower is determined by environmental conditions under which the flowers are produced and post harvest factors such as increase in the activity of peroxidase enzyme, increase in level of ethylene and due to tissue deterioration caused by microbes in the vase solution. Colonization by mycorrhizal fungi has been shown to increase the vase life of cut flowers (Wen and Chang 1995) but the mechanism involved is still unknown. Some of the reasons of having better and prolonged vase-life of cut flowers in mycorrhizal inoculated plants can be because of better vascular development by mycorrhizal fungi (Chang 1994) or due to decreased ethylene production. Besmer and Koide (1999) also attributed the increased vase-life of cut flowers of *Antirrhium majus* to the reduction of ethylene production in mycorrhizal plants. Parish (1968) suggested that increase in peroxidase activity is one of the most reliable indicators of maturity. Enhanced peroxidase activity was associated with an increase in the level of peroxides and free radicals,

which reacted with cellular constituent (Fridovich 1975). AMF inoculated plants show less increase in peroxidase activity because AMF increase the activity of antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Blilov et al. 2000). Also, these anti-oxidative enzymes constitute an important primary defense mechanism of cells against superoxide free radicals generated under stress condition (Bowler et al. 1992).

25.7.20 Physiological and Biochemical Parameters

25.7.20.1 Photosynthesis

AM fungi may function as a metabolic sink causing basipetal mobilization of photosynthates to roots thus providing a stimulus for greater photosynthetic activity (Bevege et al. 1975). Increase in activity of hormones like cytokinin and gibberellin could elevate photosynthetic rates by stomatal opening influencing ion transport and regulating chlorophyll levels (Allen et al. 1982). AMF symbiosis need carbon source from symbiotic partner synthesized by the process of photosynthesis and upto 20% of the total photoassimilates substances can be transferred to the fungal partner (Graham 2000). AMF are known to enhance the uptake of phosphorus (P) from the soil which, in turn, has an important role as energy carrier during photosynthesis.

25.7.20.2 Production of Growth Hormones

Plants with mycorrhiza exhibit higher content of growth regulators like cytokinins and auxins as compared to non mycorrhizal ones. AMF colonized roots show changes in root morphology by getting much thicker and carry fewer root hairs. Hormone accumulation in the host tissue is affected by mycorrhizal colonization with changes in the levels of cytokinins, abscissic acid, gibberellins like substances. The effect of AMF on photosynthesis and host morphology could also be hormonal. *Glomus mosseae* has been shown to synthesize phytohormones.

25.7.20.3 Alters Soil Enzyme Activity

Enzyme activity is often used as an index of total microbial activity in the soil as well as its fertility (Dhruva-Kumar et al. 1992) and is also useful in the study of changes caused in soil due to land degradation. Xyloglucanases, a hydrolytic enzyme is involved in the penetration and development of AM fungi in plant roots (Garcia-Garrido et al. 2002); esterase indicates catabolic activity in the soil, directly correlated with microbial activity of soil; phosphatases include acid as well as alkaline phosphatase that helps in release of inorganic phosphorus from

organically bound phosphorus returned to soil (Kumar et al. 1992); chitinases are known to catalyses degradation of chitin, a major component of most fungal cell wall and are also known to enhance defense mechanism, thus helps in providing protection against diseases; trehalose catalyses the hydrolysis of trehalose which is known to be a very common signal in plant symbiosis (Mellor 1992). Peroxidase enzyme activity increases in diseases and injured plant tissue but AM symbiosis is known to retard this enzyme activity by enhancing root penetration and colonization. Inoculation with AMF *G. vessiforme* enhanced soil proteinase, polyphenoloxidase, urease and saccharase activities compares with control in watermelon (Zhao et al. 2010). AM fungi are known to alter the soil enzymes activity and, thus, increase plant establishment and transport problems.

25.7.20.4 AMF in Weed Control

Sustainable system targeting *Striga* management can be achieved by the AM fungi inoculation technique. Several reports have suggested that AM fungi can change the nature/composition of weed communities in mixed culture system in a variety of ways, including changing the relative abundance of mycotrophic weeds species (colonized by AMF) and non-mycorrhizal species (noncolonized). For example, witch-weed (*Striga hermonthica*) has been found to seriously affect cereal production in many tropical countries. Infection of *Striga* resulted in a significant reduction in cereal grain yield between 20 and 100%. AMF could provide a new means of ecologically based weed management by affecting the fruiting of weed communities. According to Lendzemo (2004), *Striga* performance in the presence of AMF was negatively impacted with reduced and/or delayed germination, attachment and emergence.

25.8 Conclusion

The soil mycorrhizae are important for plant growth development and health. The use of mycorrhizae has great potential to protect plants from diseases through their biocontrol mechanism. This offers an alternative environment-friendly strategy by reducing the use of chemicals. They are the main components that enrich the soil nutrients and maintain the soil health in sustainable manner. Furthermore, they enhance the plant growth regulators, provide defense mechanism to the plants, regulate enzymatic activities, increase rate of photosynthesis and supports in bioremediations, thus acting as eco-facilitator in sustainable agriculture both in terms of production and environmental protection. Such microbial populations need systematic strategy so that their potential can be utilized in an effective way in future prospects.

References

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85:1–12

Adholeya A, Tiwari P, Singh R (2005) Commercial production of AMF inoculum and its inoculation strategies. In: Declerck S, Verma A (eds) Root-organ culture of mycorrhizal fungi. USA, pp 5–7

Ahmad F, Husain FM, Ahmad I (2011) Rhizosphere and root colonization by bacterial inoculants and their monitoring methods: a critical area in PDPR research. In: Ahmad I, Ahmad F, Pichtel J (eds) Microbes and microbial technology: agricultural and environmental technology. Springer, New York

Akashi K, Nishimura N, Ishida Y, Yokota A (2004) Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. Biochem Biophys Res Commun 323:72–78

Al-Garni SMS (2006) Increasing NaCl-salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis. Am Eur J Agric Environ Sci 1:19–26

Ali NA, Bernal MP, Ater M (2004) Tolerance and bioaccumulation of cadmium by *Phragmites australis* grown in the presence of elevated concentrations of cadmium, copper, and zinc. Aquat Bot 80:163–176

Alizadeh O, Zare M, Nasr AH (2011) Evaluation effect of mycorrhiza inoculation under drought stress condition on grain yield of sorghum (*Sorghum bicolor*). Adv Environ Biol 5:2361–2364

Al-Karaki G, McMichael B, Zak J (2004) Field response of wheat to Arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Allen MF, Moore TS, Christensen M (1982) Phytohormone changes in altered levels of gibberellin-like substances and abscisic acid in the as affected by vesicular arbuscular mycorrhizae. Plant Soil:121–130

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. Ann Bot 97:883–893

Antoun A, Prevost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddique ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht

Aronsson P, Perttu K (2001) Willow vegetation filters for wastewater treatment and soil remediation combined with biomass production. For Chron 77:293–299

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216

Ashraf M, Berge SH, Mahmood OT (2004) Inoculating wheat seedling with exopolysaccharide producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162

Auge RM, Schekel KA, Wample RL (1986) Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. New Phytol 103:107–116

Azcon-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of Arbuscular mycorrhizal fungi to the control of soil borne pathogens. In: Gianinazzi S, Schuepp H, Barea JM, Haselwandte K (eds) Mycorrhizal technology in agriculture. Birkhauser Verlag, Switzerland, pp 187–198

Bajwa R, Akhtar J, Javaid A (2003) Role of VAM in alleviating allelopathic stress of *Parthenium hysterophorus* on maize. Mycopath 1:15–30

Balestrazzi A, Botti S, Zelasco S, Biondi S, Franchin C, Calligari P, Racchi M, Turchi A, Lingua G, Berta G, Carbonera D (2009) Expression of the PsMT (A1) gene in white poplar engineered with the MAT system is associated with heavy metal tolerance and protection against 8-hydroxy-2′-deoxyguanosine mediated-DNA damage. Plant Cell Rep 28:1179–1192

Ballesteros-Almanza L, Altamirano-Hernandez J, Peña-Cabriales JJ, Santoyo G, Sanchez-Yañez JM, Valencia-Cantero E, Macias-Rodriguez L, Lopez-Bucio J, Cardenas-Navarro R,

Farias-Rodriguez R (2010) Effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes. Open Microbiol J 17:83–92
Barea JM (1991) Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. Adv Soil Sci 15:1–40
Barea JM, Palenzuela J, Cornejo P, Sánchez-Castro I, Navarro-Fernández C, Lopéz-García A et al (2011) Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. J Arid Environ 75:1292–1301
Baslam M, Goicoechea N (2012) Water deficit improved the capacity of Arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. Mycorrhiza 22:347–359
Beltrano J, Ronco MG (2008) Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus *Glomus claroideum*: effect on growth and cell membrane stability. Braz J Plant Physiol 20:29–37
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
Berta G, Sampo S, Gamalero E, Massa N, Lemanceau P (2005) Suppression of Rhizoctonia root-rot of tomato by *Glomus mossae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. Eur J Plant Pathol 111:279–288
Besmer YL, Koide RT (1999) Effect of mycorrhizal colonization and P on ethylene production by snapdragon (*Antirrhinum majus* L.) flower. Mycorrhiza 9:161–166
Bevege DI, Bowen GD, Skinner MF (1975) Comparative carbohydrate physiology of ecto and endomycorrhizas. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, New York, pp 149–175
Bhat PR, Kaveriappa KM (2007) Effect of AM fungi on the growth and nutrition uptake in some endemic Myristicaceae members of the Western ghats, India. In: Tiwari M, Sati SC (eds) The mycorrhizae: diversity, ecology and application. Daya Pub. House, Delhi, pp 295–309
Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350
Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904
Blilov IP, Bueno JA, Ocampo, Garcia-Garrido J (2000) Introduction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal *Glomus mosseae*. Mycol Res 104:722–725
Bois G, Piche Y, Fung MYP, Khasa DP (2005) Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. Mycorrhiza 15:149–158
Bowler C, Van Montagu M, Inze D (1992) Superoxide dismutase and stress tolerance. Ann Rev Plant Physiol Plant Mol Biol 43:83–116
Burdman S, Jurkevitch E, Okon Y (2000) Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture. In: Subba Rao NS, Dommergues YR (eds) Microbial interactions in agriculture and forestry. Science Publishers, Enfield, NH, pp 229–250
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
Castiglione S, Franchin C, Fossati T, Lingua G, Torrigiani P, Biondi S (2007) High zinc concentrations reduce rooting capacity and alter metallothionein gene expression in white poplar (*Populus alba* L. cv. Villafranca). Chemosphere 67:1117–1126
Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D et al (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282:209–225

Chang DCN (1994) What is the potential for management of vesicular-arbuscular mycorrhizae in horticulture? In: Robson AD, Abbott LK, Malajczuk N (eds) Management of mycorrhizas in agriculture, horticulture and forestry. Kluwer, Dordrecht, pp 187–190
Chiou TJ, Lin SI (2011) Signaling network in sensing phosphate availability in plants. Annu Rev Plant Biol 62:185–206
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 (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. https://doi.org/10.3389/fphys.2012.00091
Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902
Clijsters H, Cuypers A, Vangronsveld J (1999) Physiological responses to heavy metals in higher plants: defence against oxidative stress. Z Naturforsch CA J Biosci 54:730–734
Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182
Colla G, Rouphael 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
Daily GC (1997) Introduction: what are ecosystem services? In: Daily GC (ed) Nature's services: societal dependence on natural ecosystems. Island Press, Washington, DC, pp 1–10
De Smet I (2011) Lateral root initiation: one step at a time. New Phytol 193:867–873
De-Bello F, Lavorel S, Díaz S, Harrington R, Cornelissen JHC, Bardgett RD et al (2010) Towards an assessment of multiple ecosystem processes and services via functional traits. Biodivers Conserv 19:2873–2893
Dehne HW (1982) Interaction between vesicular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115–1119
Denton B (2007) Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi MMG 445. Basic Biotechnol 3:1–5
Dhruva-Kumar JHA, Sharha GD, Mishra RR (1992) Soil microbial population numbers and enzyme activities in relation to altitude and forest degradation. Soil Biol Biochem 24:761–767
Di Baccio D, Galla G, Bracci T, Andreucci A, Barcaccia G, Tognetti R, Sebastiani L (2011) Transcriptome analyses of *Populus x euramericana* clone I-214 leaves exposed to excess zinc. Tree Physiol 31:1293–1308
Dickie IA, Koide RT, Fayish AC (2001) Vesicular-arbuscular mycorrhizal infection of *Quercus rubra* seedlings. New Phytol 151:257–264
Dickinson NM, Pulford ID (2005) Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ Int 31:609–613
Dodd JC (2000) The role of Arbuscular mycorrhizal fungi in natural ecosystems. Outlook Agric 29:55–62
Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333
Dugassa GD, von Alten H, Schönbeck F (1996) Effects of arbuscular mycorrhiza (AM) on health of *Linum usitatissimum* L. infected by fungal pathogens. Plant Soil 185:173–182
Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280
Feber BA, Zasoki RJ, Burau RG, Urio K (1990) Zinc uptake by corn as affected by vesicular arbuscular mycorrhizae. Plant Soil 129:121–130
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

Filion MM, St. Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Foo E, Ross JJ, Jones WT, Reid JB (2013) Plant hormones in arbuscular mycorrhizal symbioses: an emerging role for gibberellins. Ann Bot 111:769–779

Fridovich I (1975) Superoxide dimutase. Annu Rev Biochem 44:147–159

Fukaki H, Tasaka M (2009) Hormone interactions during lateral root formation. Plant Mol Biol 69:437–449

Gamalero E, Berta G, Massa N, Glick BR, Lingua G (2008) Synergistic interactions between the ACC deaminase-producing bacterium *Pseudomonas putida* UW4 and the AM fungus *Gigaspora rosea* positively affect cucumber plant growth. FEMS Microbiol Ecol 64:459–467

Gamalero E, Berta G, Glick BR (2009a) The use of microorganisms to facilitate the growth of plants in saline soils. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Dordrecht, pp 1–22

Gamalero E, Lingua G, Berta G, Glick BR (2009b) Beneficial role of plant growth promoting bacteria and Arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 55:501–514

Gamalero E, Berta G, Massa N, Glick BR, Lingua G (2010) Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences on the growth of cucumber under salt stress conditions. J Appl Microbiol 108:236–245

Garcia-Garrido JM, Ocampo JA, Garcia-Romera I (2002) Enzymes in the arbuscular mycorrhizal symbiosis. In: Burns R, Dick R (eds) Enzymes in the environment: activity, ecology and application. Marcel Dekker, New York, pp 125–151

Garg N, Manchanda G (2008) Effect of arbuscular mycorrhizal inoculation on salt-induced nodule senescence in *Cajanus cajan* (Pigeon pea). J Plant Growth Regul 27:115–124

Gavito ME, Miller MH (1998) Early phosphorus nutrition, mycorrhizae development, dry matter partitioning and yield of maize. Plant Soil 199:177–186

Gerdemann JW (1975) Vesicular-arbuscular mycorrhizae. In: Torrey JG, Clarkson DT (eds) The development and function of roots. Academic Press, London, pp 575–591

Gholamhoseini M, Ghalavand A, Dolatabadian A, Jamshidi E (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, Gollotte A, Binet M, Van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530

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

Gohre V, Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115–1122

Gosling P, Hodge A, Goodlass G, Bending GC (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Graham JH (2000) Assessing cost of arbuscular mycorrhizal symbiosis in agrosystems. In: Podila GK, Donds DD (eds) Current advances in mycorrhizae research. APS Press, St Paul, pp 127–140

Graham JH, Egel DS (1988) Phytophthora root rot development on mycorrhizal and phosphorus fertilized on mycorrhizal Citrus under drought stress. New Phytol 105:411–419

Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC (2001) The importance of early season phosphorus nutrition. Can J Plant Sci 81:211–224

Grant CA, Bittman S, Montreal M, Plenchette C, Morel C (2005) Soil and fertilizer phosphorus: effects on plant P supply and mycorrhizal development. Can J Plant Sci 85:3–14

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37:395–412

Grigulis K, Lavorel S, Krainer U, Legay N, Baxendale C, Dumont M et al (2013) Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J Ecol 101:47–57

Guether M, Balestrini R, Hannah M, He J, Udvardi MK, Bonfante P (2009) Genome-wide reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. New Phytol 182:200–212

Hammond JP, White PJ (2011) Sugar signaling in root responses to low phosphorus availability. Plant Physiol 156:1033–1040

Hampp R, Mertz A, Schaible R, Schwaigerer M, Nehls U (2000) Distinction of *Araucaria angustifolia* seeds from different locations in Brazil by a specific DNA sequence. Trees 14:429–434

Hanlon MT, Coenen C (2011) Genetic evidence for auxin involvement in Arbuscular mycorrhiza initiation. New Phytol 189:701–709

Hassinen V, Vallinkoski VM, Issakainen S, Tervahauta A, Karenlampi S, Servomaa K (2009) Correlation of foliar MT2b expression with Cd and Zn concentrations in hybrid aspen (*Populus tremula x tremuloides*) grown in contaminated soil. Environ Pollut 157:922–930

Hause B, Mrosk C, Isayenkov S, Strack D (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68:101–110

Hawkins HJ, Johansen A, George E (2000) Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi. Plant Soil 226:275–285

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Huang RS, Smith WK, Yost RE (1985) Influence of vesicular-arbuscular mycorrhizae on growth, water relation and leaf orientation in *Leucaena leucocephala* (Linn.) De wit. New Phytol 99:229–243

Ivanchenko MG, Muday GK, Dubrovsky JG (2008) Ethylene–auxin interactions regulate lateral root initiation and emergence in *Arabidopsis thaliana*. Plant J 5:335–347

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

Javaid A (2009) Arbuscular mycorrhizal mediated nutrition in plants. J Plant Nutr 32:1595–1618

Jentschel K, Thiel D, Rehn F, Ludwig-Muller J (2007) Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. Physiol Plant 129:320–333

Jha DK, Sharma GD, Mishra RR (1994) Ecology of vesicular-arbuscular mycorrhiza. In: Prasad AB, Bilgrami RS (eds) Microbes and environments. Narendra Publishing House, Delhi, pp 199–208

Johansson J, Paul L, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. Microbial Ecol 18:1–13

Johnson D, Leake JR, Ostle N, Ineson P, Read DJ (2002) In situ $^{13}CO_2$ pulse-labelling of upland grasslands demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol 153:327–334

Karasawa TY, Kasahara M, Takebe (2002) Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous Arbuscular mycorrhizal fungi. Soil Biol Biochem 34:851–857

Kaur M, Mukerji KG (1999) The application of vesicular Arbuscular mycorrhizal fungi in afforestation. In: Singh A, Aneja KR (eds) From ethanomycology to fungal biotechnology. Plenum Press, New York, pp 213–224

Kaye JW, Pfleger FL, Stewart EL (1984) Interactions of *Glomus fasciculatum* and *Pythium ultimumon* green house grown Poinsettia. Can J Bot 62:1575–1579

Khalafallah AA, Abo-Ghalia HH (2008) Effect of Arbuscular mycorrhizal fungi on the metabolic products and activity of antioxidant system in wheat plants subjected to short-term water stress, followed by recovery at different growth stages. J Appl Sci Res 4:559–569

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant Biol 7:706–712

Khanday M, Bhat RA, Haq S, Dervash MA, Bhatti AA, Nissa M, Mir MR (2016) Arbuscular mycorrhizal fungi boon for plant nutrition and soil health. In: Hakeem KR et al (eds) Soil science: agricultural and environmental prospective. Springer International Publishing, Switzerland, pp 317–332

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734

Kohler A, Blaudez D, Chalot M, Martin F (2004) Cloning and expression of multiple metallothioneins from hybrid poplar. New Phytol 164:83–93

Kothari SK, Marschner H, Romheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. New Phytol 116:637–645

Kumar DJHA, Shasha GD, Mishra RR (1992) Soil microbial population numbers and enzyme activities in relation to latitude and forest degradation. Soil Biol Biochem 24:761–767

Lau JA, Lennon JT (2011) Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New Phytol 192:215–224

Lavorel S (2013) Plant functional effects on ecosystem services. J Ecol 101:4–8

Lebeau T, Braud A, Jezequel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522

Lei M, Zhu C, Liu Y et al (2010) Ethylene signalling is involved in regulation of phosphate starvation-induced gene expression and production of acid phosphatases and anthocyanin in Arabidopsis. New Phytol 189:1084–1095

Lendzemo VW (2004) The tripartite interaction between sorghum, *Striga hermonthica* and Arbuscular mycorrhizal fungi. Ph.D thesis, Wageningen University, Wageningen, The Netherlands

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

Liu RJ, Chen YL (2007) Mycorrhizology. China Science Press, Beijing, p 447

Liu J, Wu L, Wei S, Xiao X, Su C, Jiang P, Song Z, Wang T, Yu Z (2007) Effects of Arbuscular mycorrhizal fungi on the growth, nutrient uptake and glycyrrhizin production of licorice (*Glycyrrhiza uralensis* Fisch). Plant Growth Regul 52:29–39

López-Ráez JA, Verhage A, Fernández I (2010) Hormonal and transcriptional profiles highlight common and differential host responses to Arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. J Expt Bot 61:2589–2601

Ludwig-Muller J (2010) Hormonal responses in host plants triggered by Arbuscular mycorrhizal fungi. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function, 2nd edn. Springer, Dordrecht, pp 169–190

Mar Vazquez M, Cesar S, Azcon R, Barea JM (2000) Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl Soil Ecol 15:261–272

Marhavý P, Bielach A, Abas L et al (2011) Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. Dev Cell 21:796–804

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, London

Martín-Rodríguez JA, León-Morcillo R, Vierheilig H, Ocampo JA, Ludwig-Muller J, García-Garrido JM (2011) Ethylene-dependent/ethylene-independent ABA regulation of tomato plants colonized by Arbuscular mycorrhiza fungi. New Phytol 190:193–205

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, Barea JM, Azcon R (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

Matamoros MA, Loscos J, Dietz K, Aparicio-Tejo PM, Becana M (2010) Function of antioxidant enzymes and metabolites during maturation of pea fruits. J Exp Bot 61:87–97

Maya MA, Matsubara Y (2013) Influence of arbuscular mycorrhiza on the growth and antioxidative activity in cyclamen under heat stress. Mycorrhiza 23:381–390

McFarland J, Ruess R, Keilland K, Pregitzer K, Hendrick R, Allen M (2010) Cross-ecosystem comparisons of in situ plant uptake of amino acid-N and $NH4^{+}$. Ecosystems 13:177–193

Meddich A, Jaiti F, Bourzik W, Asli AE, Hafidi M (2015) Use of mycorrhizal fungi as a strategy for improving the drought tolerance in date palm (*Phoenix dactylifera*). Sci Hortic 192:468–471

Meding SM, Zasoski RJ (2008) Hyphal-mediated transfer of nitrate, arsenic, cesium, rubidium, and strontium between arbuscular mycorrhizal forbs and grasses from California oak woodland. Soil Biol Biochem 40:126–134

Mellor RB (1992) Is trehalose asymbiotic determinant in symbiosis between higher plants and microorganisms? Symbiosis 12:113–129

Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol 12:563–569

Miransari M, Bahrami HA, Rejali F, Malakouti MJ (2008) Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biol Biochem 40:1197–1206

Mosse FE (1973) Advance in the study of vesiculararbuscular mycorrhizae. Ann Rev Phytopathol 72:1125–1132

Mrnka L, Kuchar M, Cieslarova Z, Matejka P, Szakova J, Tlustos P, Vosatka M (2012) Effects of endo- and ectomycorrhizal fungi on physiological parameters and heavy metals accumulation of two species from the family Salicaceae. Water Air Soil Pollut 223:399–410

Muday GK, Rahman A, Binder BM (2012) Auxin and ethylene: collaborators or competitors? Trends Plant Sci 17:181–195

Mukherjee A, Ané JM (2011) Germinating spore exudates from Arbuscular mycorrhizal fungi: molecular and developmental responses in plants and their regulation by ethylene. Mol Plant Microbe Interact 24:260–270

Najafi A, Ardakani MR, Rejali F, Sajedi N (2012) Response of winter barley to co-inoculation with Azotobacter and Mycorrhiza fungi influenced by plant growth promoting rhizobacteria. Ann Biol Res 3:4002–4006

Nicolson TH (1967) Vesicular-arbuscular mycorrhizal: a universal plant symbiosis. Sci Prog (Oxf) 55:561

Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS (2013) Responses of root architecture development to low phosphorous availability: a review. Ann Bot 112:391–408

Nunes JLD, de Souza PVD, Marodin GAB, Fachinello JC (2010) Effect of arbuscular mycorrhizal fungi and indole butyric acid interaction on vegetative growth of 'Aldrighi' peach rootstock seedlings. Cienc Agrotecnol 34:80–86

Ordookhani K, Khavazi K, Moezzi A, Rejali F (2010) Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. Afr J Agric Res 5:1108–1116

Oueslati O (2003) Allelopathy in two durum wheat (*Triticum durum* L.) varieties. Agric Ecosyst Environ 96:161–163

Ouziad F, Wilde P, Schmelzer E, Hildebrandt U, Bothe H (2006) Analysis of expression of aquaporins and Na^{+}/H^{+} transporters in tomato colonized by arbuscular mycorrhizal fungi and affected by salt stress. Environ Exp Bot 57:177–186

Parish RW (1968) Studies on senescing tobacco leaves disc with special reference to peroxidase. The effect of cutting and inhibition of nucleic acid and protein synthesis. Planta 82:1–13

Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39

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

Quilambo OA (2000) Functioning of peanut (*Arachis hypogaea* L.) under nutrient deficiency and drought stress in relation to symbiotic associations. Ph.D thesis, University of Groningen, The Netherlands. Van Denderen B.V., Groningen. ISBN:903671284X

Rajkumar M, Sandhya S, Prasad M, Freitas H (2012) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol Adv 30:1562–1574

Ramos-Zapata JA, Orellana R, Allen EB (2006) Establishment of *Desmoncus orthacanthos Martius* (Arecaceae): effect of inoculation with Arbuscular mycorrhizae. Rev Biol Trop 54:65–72

Rani P, Aggarwal A, Sharma D (2001) Improvement in biomass yield of *Prosopis cineraria* through VAM. *Rhizobium* sp. and *Trichoderma harzianum*. Adv Plant Sci 14:593–596

Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

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

Rouached H, Arpat AB, Poirier Y (2010) Regulation of phosphate starvation responses in plants: signaling players and cross-talks. Mol Plant 3:288–299

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress: new perspectives for molecular studies. Mycorrhiza 13:309–317

Ruiz-Lozano JM, Aroca R (2010) Host response to osmotic stresses: stomatal behaviour and water use efficiency of arbuscular mycorrhizal plants. In: Koltai H, Kapulnik Y (eds) Arbuscular mycorrhizas: physiology and function. Springer, Netherlands, pp 239–256

Ruız-Lozano JM, Azcon R, Gomez M (1995) Effects of arbuscular-mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Appl Environ Microbiol 61:456–460

Ryan MH, Ash JE (1996) Colonisation of wheat in southern New South Wales by vesicular-arbuscular mycorrhizal fungi is significantly reduced by drought. Aust J Exp Agric 36:563–556

Safir GR, Nelson CE (1985) VA-mycorrhizas plant and fungal water relations. In: Molina R (ed) Proceedings of 6th North American conference on mycorrhiza, Corvallis, p 471

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21. http://astonjournals.com/lsmr

Sakakibara H (2006) Cytokinins: activity, biosynthesis, and translocation. Annu Rev Plant Biol 57:431–449

Salamanca CP, Heera MA, Barea JM (1992) Mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified Mediterranean ecosystems. Agronomie 12:869–872

Sannazzaro AI, Ruiz OA, Alberto EO, Menendez AB (2006) Alleviation of salt stress in *Lotus glaber* by *Glomus intraradices*. Plant Soil 285:279–287

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

Sato A, Miura K (2011) Root architecture remodeling induced by phosphate starvation. Plant Signal Behav 6:1122–1126

Schenk NC (1981) Can mycorrhizae control root diseases? Plant Dis 65:230–234

Schliemann W, Ammer C, Strack D (2008) Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochemistry 69:112–146

Schellenbaum L, Muller J, Boller T, Wiemken A, Schüepp H (1998) Effects of drought on non – mycorrhizal and mycorrhizal maize: changes in the pools of non – structural carbohydrates, in the activities of invertase and trehalase, and in the pools of amino acids and imino acids. New Phytol 138:59–66

Sebastiani L, Scebba F, Tognetti R (2004) Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides x maximowiczii*) and I-214 (*P. x euramericana*) exposed to industrial waste. Environ Exp Bot 52:79–88

Selvakumar G, Thamizhiniyan P (2011) The effect of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on the growth and yield of chilli (*Capsicum annuum* L.) under salinity stress. World Appl Sci J 14:1209–1214

Sharda JN, Koide RT (2010) Exploring the role of root anatomy in P-mediated control of colonization by arbuscular mycorrhizal fungi. Botany 88:165–173

Shaul-Keinan O, Gadkar V, Ginzberg I et al (2002) Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices*. New Phytol 154:501–507

Sheng M, Tang M, Chen H, Yang BW, Zhang FF, Huang YH (2009) Influence of arbuscular mycorrhizae on the root system of maize plants under salt stress. Can J Microbiol 55:879–886

Shinde SK, Shinde BP, Patale SW (2013) The alleviation of salt stress by the activity of AM fungi in growth and productivity of onion (*Allium cepa* l.) plant. Int J Life Sci Pharma Res 3:11–15

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

Smith SE, Read DJ (1997a) Mycorrhizal symbiosis, 2nd edn. Academic Press, London, p 605

Smith SE, Read DJ (1997b) Vesicular-arbuscular mycorrhizas. In: Mycorrhizal symbiosis, 2nd edn. Academic Press, London, pp 9–160

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic Press, London

Smith SE, Facelli E, Pope S, Smith F (2010) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20

Subramanian KS, Charest C (1999) Acquisition of N by axternal 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

Subramanian KS, Santhanakrishnan P, Balasubramanian P (2006) Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci Hortic 107:245–253

Sukumar P, Legué V, Vayssières A et al (2013) Involvement of auxin pathways in modulating root architecture during beneficial plant-microorganism interactions. Plant Cell Environ 36:909–919

Sylvia, DM, Williams SE (1992) Vesicular-arbuscular mycorrhizae and environmental stress. In: Lindermann RG, Bethlenflavay GJ (eds) Mycorrhizae in sustainable agriculture, American Society of Agronomy. Special Publication No. 54, Madisn, WI, pp 101–124

Symanczik SJ, Blaszkowski J, Chwat G, Boller T, Wiemken A, Al-Yahya'ei MN (2014) Three new species of arbuscular mycorrhizal fungi discovered at one location in a desert of Oman: *Diversispora omaniana, Septoglomus nakheelum* and *Rhizophagus arabicus*. Mycologia 106:243–259

Takacs D, Radimszky L, Nemeth T (2005) The arbuscular mycorrhizal status of poplar clones selected for phytoremediation of soils contaminated with heavy metals. Z Naturforsch CA J Biosci 60:357–361

Takeda N, Kistner C, Kosuta S, Winzer T, Pitzschke A, Groth M et al (2007) Proteases in plant root symbiosis. Phytochemistry 68:111–121

Thewys T, Witters N, Meers E, Vangronsveld J (2010) Economic viability of phytoremediation of a cadmium contaminated agricultural area using energy maize. Part II: economics of anaerobic digestion of metal contaminated maize in Belgium. Int J Phytorem 12:663–679

Tobar RM, Azcon R, Barea JM (1994) Improved nitrogen uptake and transport from ^{15}N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water stressed conditions. New Phytol 126:119–122

Todeschini V, Franchin C, Castiglione S, Burlando B, Biondi S, Torrigiani P, Berta G, Lingua G (2007) Responses to copper of two registered poplar clones inoculated or not with Arbuscular mycorrhizal fungi. Caryologia 60:146–155

Tognetti R, Cocozza C, Marchetti M (2013) Shaping the multifunctional tree: the use of Salicaceae in environmental restoration. Forest 6:37–47

Torelli A, Trotta A, Acerbi L, Arcidiacono G, Berta G, Branca C (2000) IAA and ZR content in leek (*Allium porrum* L.) as influenced by P nutrition and arbuscular mycorrhizae, in relation to plant development. Plant Soil 226:29–35

van der Lelie D, Taghavi S, Monchy S, Schwender J, Miller L, Ferrieri R, Rogers A, Wu X, Zhu W, Weyens N, Vangronsveld J, Newman L (2009) Poplar and its bacterial endophytes: coexistence and harmony. Crit Rev Plant Sci 28:346–358

Vanstraelen M, Benková E (2012) Hormonal interactions in the regulation of plant development. Annu Rev Cell Dev Biol 28:463–487

Vivas A, Azcon R, Biro B, Barea JM, Ruiz-Lozano JM (2003) Influence of bacterial strains isolated from lead-polluted soil and their interactions with arbuscular mycorrhizae on the growth of *Trifolium pratense* L. under lead toxicity. Can J Microbiol 49:577–588

Wen CL, Chang DCN (1995) Effects of temperature and Glomus sp. on the cut flower quality of micropropagated *Gerbera jamesoni*. Mem Coll Agric Natl Taiwan Univ 35:75–91

Werner T, Nehnevajova E, Köllmer I et al (2010) Root-specific reduction of cytokinin causes enhanced root growth, drought tolerance, and leaf mineral enrichment in *Arabidopsis* and tobacco. Plant Cell 22:3905–3920

Willis A, Rodriguesb BF, Harrisa PJC (2013) The ecology of Arbuscular mycorrhizal fungi. Crit Rev Plant Sci 32:1–20

Wu QS, Xia RX (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425

Wu QS, Zou YN, Xia RN (2006) Effect of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. Eur J Soil Biol 42:166–172

Wu QS, Li GH, Zou YN (2011a) Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (*Prunus persica* l. Batsch) seedlings. J Anim Plant Sci 21:746–750

Wu Q, Zou Y, He X (2011b) Differences of hyphal and soil phosphatase activities in drought-stressed mycorrhizal trifoliate orange (*Poncirus trifoliata*) seedlings. Sci Hortic 129:294–298

Wu QS, Srivastava AK, Zou YN (2013) AMF induced tolerance to drought stress in citrus: a review. Sci Hortic 164:77–87

Yadav K, Singh N, Aggarwal A (2011) Influence of arbuscular mycorrhiza (AM) fungi on survival and development of micropropagated *Acorus calamus* L. during acclimatization. J Agric Technol 7:775–781

Yang Y, Tang M, Sulpice R, Chen H, Tian S, Ban Y (2014) Arbuscular mycorrhizal fungi alter fractal dimension characteristics of *Robinia pseudoacacia* L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J Plant Growth Regul 33:612–625

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

Zhang HH, Tang M, Chen H, Zheng C, Niu Z (2010) Effect of inoculation with AM fungi on lead uptake, translocation and stress alleviation of *Zea mays* L. seedlings planting in soil with increasing lead concentrations. Eur J Soil Biol 46:306–311

Zhang YF, Wang P, Yang YF, Bi Q, Tian SY, Shi XW (2011) Arbuscular mycorrhizal fungi improve reestablishment of *Leymus chinensis* in bare saline–alkaline soil: implication on vegetation restoration of extremely degraded land. J Arid Environ 75:773–778

Zhao M, Li M, Liu RJ (2010) Effect of Arbuscular mycorrhizae on microbial population and enzyme activity in explant soil used for watermelon production. Int J Eng Sci Technol 2:17–22

Zhu X, Song F, Xu H (2010) Influence of arbuscular mycorrhiza on lipid peroxidation and antioxidant enzyme activity of maize plants under temperature stress. Mycorrhiza 20:325–332

Zolfaghari M, Nazeri V, Sefidkon F, Rejali F (2013) Effect of arbuscular mycorrhizal fungi on plant growth and essential oil content and composition of *Ocimum basilicum* L. Iran. J Plant Physiol 3:643–650

Chapter 26
An Overview on Orchid Endophytes

Bijaya Pant, Sujit Shah, Roshni Shrestha, Sushma Pandey, and Pusp Raj Joshi

Abstract Orchids, one of the most beautiful and diverse plant species in the nature, are a bit of a mystery for their seeds lack endosperm and they must depend on endophytes for germination, growth and adaptation. Naturalists and even the general public are drawn to orchids for their ornamental, medicinal and food value. In their keenness to harvest them, collectors have rendered many orchid species threatened or even endangered. Recent research into orchids, which are mycohetetrophic plants, has focused on isolation and identification of the mycorrhizal and non-mycorrhizal endophytes that directly or indirectly contribute to the growth and development of orchids as well as the production of valuable secondary metabolites. This article considers both the role such endophytes play and explains how such symbiotic partner scan be used in the plant tissue culture technique to help conserve and even commercialize various species of orchid.

26.1 Introduction

Orchids are a beautiful gift of nature whose evolution, survival and adaptation is somewhat of a mystery, for orchids lack endosperm and are dependent on endophytes for germination, growth and adaptation. Because orchids have considerable ornamental, food and medicinal value, the demand for them in national and international markets is high (Bulpitt 2005; Pant 2013). Specifically, they are used in Chinese and Ayurvedic medicine to cure different diseases, often, in some least developed countries at least, as part of a repertoire of medicinal plants upon which people are dependent for primary healthcare (Pant and Raskoti 2013). Almost all parts of the orchid plant, particularly the roots, leaves, and pseudo-bulbs, have the potential to cure diseases like rheumatism, bronchitis, nervous disorders, piles, inflammation, microbial infection and cancer (Gutiérrez 2010).

Orchid propagate in nature through seed germination and vegetative propagation, however both of the propagation process are very slow, and the rate of successful natural propagation process is very low, <5% (Vij 2002).

B. Pant (✉) • S. Shah • R. Shrestha • S. Pandey • P.R. Joshi
Central Department of Botany, Tribhuvan University, Kathmandu, Nepal
e-mail: bijayapant@gmail.com

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_26

The physiology of seed germination in orchids is most interesting phenomenon in biology. For the germination of minute orchid seed in nature, seeds must enter into the symbiotic interaction with species specific Basidiomycetes fungus symbionts. Once the fungus penetrate the orchid seed, which lacks endosperm, and has no reserved food, get the nutrients from the fungus and initiates the germination process. The germinating seed nourished from the colonizing fungal symbiont, then forms protocorm, a unique embryonic structure, made up of a mass of the cell found in no other flowering plants. Though this symbiosis between orchid and fungus for mutual benefit is yet to be understood in details, it seems that orchid controls and regulate the timing and degree of fungal association providing the sufficient reasons for the fungus to colonize and be associated with the orchid plant (Arditti and Predgeon 1997).

Since in the symbiotic germination, seed takes long time for their germination and any disturbance in the habitat or physical environment destroy the whole population, plant tissue culture technique have been accepted and applied as a potential alternative method for the mass propagation of orchids, both for conservation and commercial production (Arditti and Ernst 1984; Pongener and Deb 2011).

The orchids were the first flowering plants to be commercially propagated *in vitro* through the plant tissue culture techniques which started in the early sixties. Morel produced a large clone of a virus-free plantlet from the *Cymbidium* orchid using shoot-tip culture (Arditti 1967). Soon after, 22 genera of orchids were propagated through shoot tip, root tip and embryo culture (Murashige 1974). In nature, it takes years for the seedling of an orchid to become an adult plant. In fact, growth is so slow that some endangered, indigenous species of orchids may become extinct. Not surprisingly, the Orchidaceae family is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Cribb et al. 2003). The conservation of the orchid is an important task to protect the concomitant losses of biodiversity, wild beauty and their therapeutics potential (Pant et al. 2016).

The mass propagation of orchids can be achieved using plant tissue culture techniques. Modified forms of media and different chemical compounds, gelling agent, organic and inorganic salts and cofactors are used to propagate orchids *in vitro* (Deb 2008; Hossain 2009; Pant and Thapa 2012; Pradhan et al. 2014, 2016). Light of different intensity and colour also play a significant role in plant tissue culture (Baque et al. 2011). The isolation and identification of endophytes from various orchid species in nature could be used as a biological tool to accomplish the same end. Endophytes play a significant role in plant growth and development, nutrient supply and immune defence (Feng et al. 2002; Giri et al. 2003; Shimura et al. 2007). The use of endophtyes is a promising practice that could help to overcome some challenges face by *in vitro* plantlets during acclimatization, such as high mortality rates, poor growth and poor immunity.

Some species of orchids found in Nepal from which diverse endophytes have been isolated were identified in a recent research paper (Liu et al. 2010; Ma et al. 2015) (Fig. 26.1).

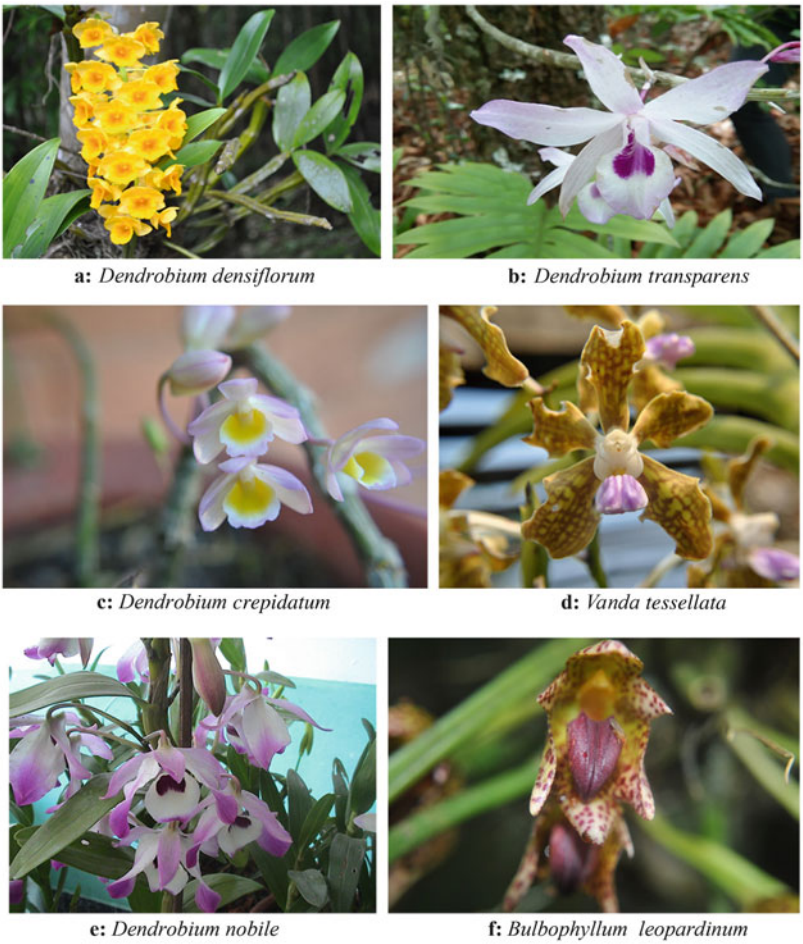


Fig. 26.1 (**a-f**) Some of the orchid species found in Nepal from which diverse endophytes have been isolated

26.2 Role of Orchid Microbes

Mycorrhizal association is an important phenomenon that emerged during the course of evolution; indeed, it helped both plants and microbes to survive. Fossil evidence suggests that this relationship existed over 400 million years ago in the tissue of the first terrestrial plant (Pirozynski 1989). In nature, most plants function in symbiotic association with microbes; in fact, some depend on their relationships with bacteria or fungi for their very survival. Some of the bacteria that show symbiosis with orchids are *Streptomyces*, *Bacillus*, *Erwinia*, *Pseudomonas*, *Flavobacterium*, *Sphingomonas paucimobilis* ZJSH1, and *Streptosporangium oxazolinicum* (Tsavkelova et al. 2007; Yang et al. 2008).

Evidence of the significant role of bacteria in the formation and establishment of mycorrhizal association was demonstrated by Duponnois and Garbaye (1991). They showed that *Pseudomonas flurorescens* BBC6 help form ectomycorrhiza and term them "mycorrhiza helper bacteria" (MHB). In conditions of stress, such MHB secretes the secondary metabolite or biomolecules that help in fungal spore germination, mycelia growth, root colonization, and the development of well-developed root systems (Frey-Klett et al. 2007; Giri et al. 2005). The benefits go both ways: while mycorrhizal-associated plants get sufficient nutrients such as phosphate and other minerals from fungi, fungi get sugar in return (Al-Karaki and Al-Raddad 1997). Endophytic fungi are localized in various plant tissues and form rich and diverse fungi community (Gennaro et al. 2003). They colonize in internal plant tissue, especially velamen layers, cortical cell layers, hyphae and endodermal passage cells, without causing harm to the host (Moreira and Dos Santos Isaias 2008).

One of the functions of mycorrhizal association that evolved is to fight against pathogens, hence providing immunity to the plants (Feng et al. 2002). There is also evidence that microbial association helps plants to overcome abiotic stress such as drought, salinity, oxidative stress (Giri et al. 2003). For example, the production of biomolecules by *Rhizobia* such as aminocyclopropane-1-carboxylate ACC mitigates plant stress (Macdonald and Chandler 1981).

Recent research on the isolation and identification of endophytes from host plants can conserve plants and build our understanding about how plants adapt to their native environments. In this regard, the discovery of *Piriformospora indica* has revolutionized the field of plant and microbes interaction because it can be cultured axenically and interacts with wide a range of plant species. In fact, it is considered as an ideal endophyte for research and field application (Varma et al. 1999, 2012; Prasad et al. 2013; Gill et al. 2016).

Endophtyes play an important role in plant tissue culture: as illustrated in Fig. 26.2 they function as bioregulators, biofertilizers and bioprotectors.

26.2.1 Biofertilization: The Role of Endophytes in Nutrient Supply

For proper growth and development, plants obtain both macro- and micronutrients from the soil, water and the atmosphere. While these nutrients are required in only in trace amounts, their depletion from the surrounding environment can directly or indirectly affect the growth and development of plant. Indeed, trace nutrients are regarded as a limiting factor for plant growth and development. Mycorrhizal association helps plants uptake minerals such as carbon, phosphorous, and nitrogen because their hyphae penetrate the soil, thereby increasing the surface area for the nutrient uptake and enabling a root system to uptake nutrients from beyond the zone of depletion.

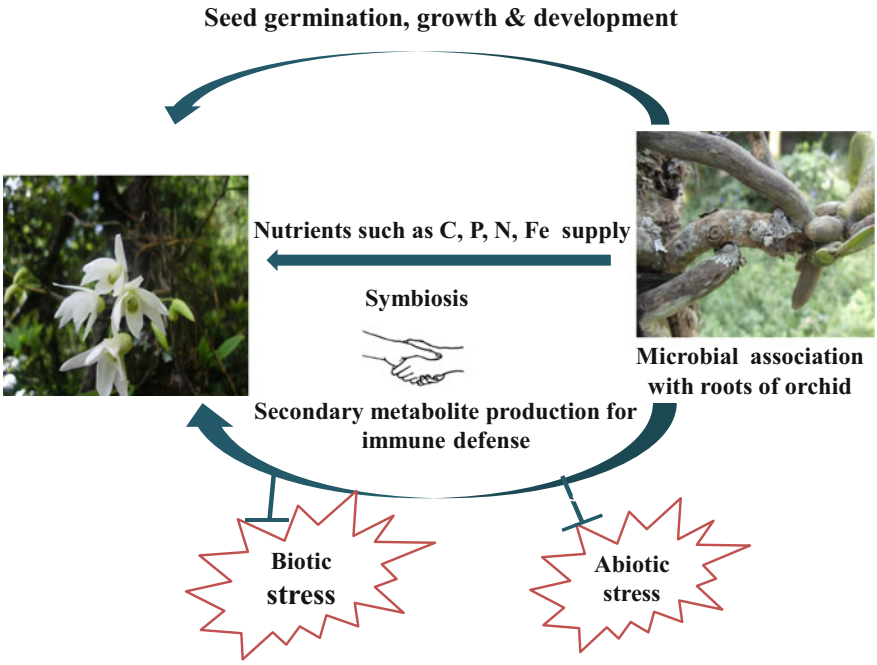


Fig 26.2 The symbiotic relationship between an orchid plant and endophytic fungi. Endophtyes acts as a biological tool promoting growth and development (bioregulation), nutrient uptake (biofertilization) and production of phytohormone (bioprotection)

Carbon is an important nutrient for plant development and growth. Orchids are grouped into three categories based on carbon nutrition: fully autotrophic, fully mycoheterotrophic, and partially mixotrophic (Zimmer et al. 2007). Generally, achlorophyllous mycoheterotrophic orchids are considered "parasitic or cheating orchids" as they are entirely dependent on partner endophytes for their nutrient supply and growth. The host endophytes do not benefit from such orchids. For example, *Achlorophyllous epipogium*, a mycoheterotrophic orchid rhizome colonized by an endophyte *Inocybe* species which forms clamp and dolipores, provide a significant amount of carbon to a plant (Roy et al. 2009).

Because orchids seeds lack the endosperm which provides the carbon needed for the germination and growth of seeds, most orchids depend on microbes to uptake carbon (Gennaro et al. 2003; Zimmer et al. 2007). Carbon is mainly deposited in the form of starch in the roots of a plant. Alexander and Hadley (1985) were able to track the movement of carbon alone from fungi to the host plant, *Goodyera repens* using a radio-labeled insoluble carbohydrate as the carbon-only nutrient for the external mycelium of the fungi. They were able to show that the infected protocorm obtained the radio-labeled carbon from external mycelium. After a certain stage of development, however, that transfer ceased. Clearly, an endophyte can help an orchid meets it's need for carbon for seed germination and protocorm development.

Similar findings were reported by Stöckel et al. (2014), with the radio-labeled ^{13}C and ^{15}N assay. They studied the carbon and nitrogen flow from the fungus to the host plant, selected four orchids *Neottia nidus-avis*, *Epipactis helleborine*, *Serapias parviflora*, *Pseudorchis albida*. They were able to find the specific association of *N. nidus-avis*, *E. helleborine* with ectomycorrhizal fungi and that of *S. parviflora*, *P. albida* with saprophytic rhizoctonia fungi. The flow or gain of carbon and nitrogen from the fungus to the protcorms of *N. nidus-Avis*, *E. helleborine* was significantly greater than that of adult. In contrast, the flow of carbon and nitrogen from saprotrophic fungi to the protocorms of *S. parviflora, P. albida* significantly lesser than that ectomycorrhizal fungus associated with *N. nidus-avis, E. helleborine*. However, their finding was contradictory to the various finding related to rhizoctonia being growth promoter of orchid seedling. Similarly, Gebauer and Meyer (2003) showed the significant carbon and nitrogen uptake by autotrophic *Cephalanthera damasonium* from fungal association. Similarly, Wang et al. (2013) were able to demonstrate that mycorrhizal association plays a role in carbon acquisition and utilization by *Dendrobium officinale*.

Phosphorous is another major nutrient plants require. It plays a role in various functions such as energy metabolism, respiration, photosynthesis and bimolecular nucleic acid. In soil, phosphorous is present in three forms: inorganic (orthophosphate and polyphosphate), organic (organically bound phosphate) and phytates (Schachtman et al. 1998). The inorganic form, which comprises about 15% of the total, is freely available, but the remaining 80−85, 5−7% are phyates, is immobile and thereby unavailable to plants. Plants can uptake the phosphorous by two distinct modes: through their own transport system or through mycorrhizal association (Li et al. 2013). As is the case with all nutrients, phosphorous can be better absorbed by a fungi-colonized root because the fungi increase the surface area for absorption and extend beyond the nutrient-depleted area surrounding a plant. *P. indica* is an endophyte well known for phosphate solubilization (Malla et al. 2004). It has acidic and alkaline phosphates that convert the organic form of phosphate into the inorganic form (Das et al. 2014), thereby providing soluble phosphorous to a plant (Shahollari et al. 2005). Similarly, phosphate solubilizing bacteria secrete phosphatase enzymes to solubilize insoluble phosphate (Kim et al. 1998). These bacteria, which include *Bacillus*, *Streptomyces luteogriseus* and *Pseudomonas*. Matsuoka et al. (2013) also foster the production of siderophores. Microbial association not only helps in nitrogen uptake, it promotes the formation of nitrogenous compounds and nitrogen metabolism. The role of microorganisms is especially crucial as plants cannot absorb nitrogen from the atmosphere, where it is plentiful, because they lack a nitrogenase enzyme system. Microbes, however, do have such a system and can fix nitrogen.

While both the ammonium and nitrate forms of nitrogen are freely available to plants (Miller and Cramer 2005), most plants prefer the ammonium form (Scherer and Ahrens 1996). They also use organic forms of nitrogen such as low molecular weight amino acids (Chapin et al. 1993). Nitrogen fixation is carried out by both nitrogen-fixing bacteria and arbuscular fungi. Already, extensive research work has been carried out on the nitrogen-fixing bacteria found in legumes. In particular, the symbiotic diazotrophs, *Rizobium* and *Frankia* have been studied in great detail.

While research on the presence of such bacteria in orchids is, in contrast, in its nascent stages, Dighe et al. (1986) did report the presence of the nitrogen-fixing bacteria *Azotobacter* in an epiphytic orchid, *Vanda tessellate*. Moreover, they were able to show that microbial association enables the production of auxin.

26.2.2 Bioregulation: Role of Orchid Endophytes in Growth and Development

Plant hormones such as auxin, cytokinin, gibberelline, and ethylene play an important role in plant growth and development, and microbial association helps in the production and regulation of these and other phytohormones by regulating their pathway. Several studies show the importance of endophtyes in the germination of orchid seeds and the growth of protocorns, seedlings and adult plants. Xu and Guo (1989) reported the association of two different species of fungi with *Gastrodia elata* at different stages of the plant life cycle, while Leake (1994) suggested that more than 100 species of orchids depend on endophytic fungi throughout their lifetime. *Rhizoctonia zeae* isolated from *Vanda coerulea* showed it plays a significant role in promoting *in vitro* seed germination and seedling growth as well as in increasing the survival rate during acclimatization (Aggarwal et al. 2012). Orchid seed germination and protocorm development is also dependent on the effects of *P. indica* (Varma et al. 2013). These facts suggest that endophytes are crucial for plant growth and development and that more research into the role of endophytes and their co-evolution with orchids needs to be done (Rasmussen and Rasmussen 2009) (Fig. 26.3).

Microbial association helps in the production of auxin, a hormone which plays an important role in organogenesis, cell expansion, cell division, cell differentiation and gene regulation. Endophytes such as *Streptomyces, Bacillus, Pseudomonas, Burkholderia, Erwinia* and *Nocardia* colonized in *Paphiopedilum* roots and endophytes such as *Pseudomonas*, *Flavobacterium, Stenotrophomonas, Pantoea, Chryseobacterium, Bacillus, Agrobacterium, Erwinia, Burkholderia* and *Paracoccus* colonized in the roots of *Pholidota articulata* Lindl orchid helped produce auxin (Tsavkelova et al. 2007). Endophytic bacteria of the strains *Bacillus*, *Burkholderia*, *Enterobacteria* and *Curtobacteria* isolated from *Cattelya walkeriea*, an endemic and threatened orchid species found in Brazil, were shown to play a significant role in auxin production, too. The interaction of these rhizobacteria, especially *Bacillus* and *Enterobacter* species, with seeds and seedlings resulted in high rates of germination and growth and better acclimatization (Galdiano Junior et al. 2011).

Orchid endophytes help plants in other ways, too. Two endophytic bacteria isolated from *Cymbidium eburneum*, *Paenibacillus lemtimorbus* and *Paenibacillus macercus*, have the potential to synthesize indole acetic acid. These endophytes were able to increase the root length, shoot length and number of plantlets and to promote survival during acclimatization. Similarly, the *Sphingomonas paucimobilis* ZJSH1 associated with *Dendrobium officinale* helps in the production of phytohormones such as auxin, zeatin and ABA (Yang et al. 2014a).

Fig. 26.3 Root induction of in vitro grown *Cymbidium aloifolium (protocorm stage)* when treated with 10% fungal elicitor for 30 days (**a**) compared with the in-vitro grown *Cymbidium aloifolium* (protocorm stage) without fungal elicitor treatment for 30 days (**b**)

26.2.3 Immune Modulator: The Role Orchid Endophytes as a Defense Mechanism

Plants have immune systems that enable them to address environmental challenges. Certain bacteria and fungi help to modulate that system. Specifically, endophytes such as bacteria or fungi help overcome both biotic and abiotic stresses because they help produce the phytohormones necessary to resist those stresses. Biotic stresses are due to disease-causing pathogens present in the environment and abiotic stresses include salinity, drought, high acidity, extreme cold and heats, free radicals and reactive oxygen species.

Orchid extracts contain various important compounds like flavonoid, Bibenzyl derivative; Coeloginanthridin, Coeloginanthrin;2,4,7-Trihydroxy-9,10-Dihydrophenanthrene; 3,7-dihydroxy-2,4,8-Trimethoxyphenanthrene; Coelonin; Stilbenoids; Sesquiterpene derivatives; 3,7-Dihydroxy-2,4-dimethoxyphenanthren which are referred as secondary metabolites (He et al. 2006; Majumder et al. 1999; Majumder and Bandyopadhyay 2010; Moin et al. 2012; Zeghad and Merghem 2013). These compounds possess antioxidant, antimicrobial as well as free radical

scavenging properties (Paudel et al. 2015; Chand et al. 2016). In fact, plants, in general, are a good source of catalase, glutathione reductase, and ascorbate-glutathione peroxides, all antioxidants which regulate reactive oxygen species and other free radicals in the biological system (Almeselmani et al. 2006). Plants also form superoxide and hydrogen peroxide, compounds that acts as signaling molecules and help activate various proteins (Tripathy and Oelmüller 2012). Since the accumulation of such reactive oxygen species can lead to DNA damage, protein dysfunction and cell death (van Breusegem and Dat 2006), it is important that their production be regulated. Antioxidants and secondary metabolites can do this. In fact, these compounds play an important role in DNA repair, the regulation of free radicals and reactive oxygen species, the activation of protein and the inhibition of pathogens, making the contribution that endophytes make to the production of such compounds an important issue to explore.

Endophytes can overcome not just biotic stress, but also abiotic ones, as the isolation of a novel compound from orchid endophytes shows: *Pestalotiopsis*, an endophyte isolated from *Dendrobium officinale*, has both antifungal and antioxidant properties. It controlled microbes such *Candida, Crytococcus neoformans, Trichophyton rubrum* and *Aspergillus fumigatus*. AM fungi like *Glomus fasciculatum* and *G. macrocarpum* also play a key role in overcoming abiotic stresses like salinity. *P. indica* is an endophyte that helps to overcome abiotic stresses. *Hordeum vulgare* cv whose roots are colonized by *P. indica* show more growth and resistance to salinity than do controls (Baltruschat et al. 2008), and *Arabidopsis* sp. and *Brassica campestris* sp. whose roots are colonized by *P. indica* were more able then controls to overcome drought and increase their survival and growth rates (Sherameti et al. 2008; Sun et al. 2010). Further research into how microbe orchid interaction helps plants address abiotic stresses have tremendous potential.

Interestingly, many research over the years have concluded that endophytes can encounter the human pathogen (Wu et al. 2016). For example, endophytes, which have antioxidant properties, can induce the apoptosis of cancer cell lines. Indeed fungi can synthesize most known natural compound taxols (Yang et al. 2014b), compounds which are used to treat cancer. Other compounds like alkaloids, terpenoids, isocoumarin derivatives, coumarins, chromones, quinones, semiquinones, peptides, xanthones and xanthone derivatives; phenolic compound and lactones, all of which can be synthesized by endophytes, also have the potential to cure diseases (Nicoletti and Fiorentino 2015; Chen et al. 2016).

26.3 Molecular and Cellular Interaction Behind Mycorrhizal Establishment

Researchers are interested in how plants are able to recognize compatible or even beneficial partners from among the vast pool of diverse microbes present in nature. Decades of research into this ability has discovered that fine tuning and molecular coordination between plants and microbes is the key. The orchid fascinates not just humans and pollinators but also microorganisms when it secretes volatile

compounds that give-off a strong aroma. It can be speculated that microorganisms are directly or indirectly involved in the production of such compounds and which may be one factor that promotes high reproduction successes or fitness and thereby enable the orchid to survive in a challenging environment.

It is, however, a matter of debate whether or not endophytes increase the reproduction and fitness of orchids by interacting with flowers. Jacquemyn et al. (2013) were able to isolate microorganisms such as the bacteria *Acinetobacter, Bacillus, Curtobacterium, Dermacoccus, Enterobacter, Erwinia, Frigoribacterium, Leuconostoc, Microbacterium, Methylobacterium, Paenibacillus, Pectobacterium, Plesiomonas, Pseudomonas, Serratia, Sphingomonas, Staphylococcus, Tatumella,* and *Terrimonas* and fungi such as *Cryptococcus* from the floral nectar of three *Epipacti* species, *E. microphylla*, *E. muelleri* and *E. palustris*.

Some researchers have proposed that flavonoid is the signaling molecule responsible for such microbe–plant interactions. The phenolic produced by microbes also works as a signaling molecule (Mandal et al. 2010). During a microbe–plant interaction, a series of characteristic complex morphogenetic changes take place in the structure of the fungus which has colonized a host. It may change its germination of spores, hyphal differentiation, or arbuscular formation (root penetration) (Koide 1992; Bonfante and Perotto 1995).

During mycorrhizal association, nutrients such as carbon, nitrogen and phosphate flow from soil to fungus and fungus to plant or vice-versa. Many reports suggest that from fungi require carbon from plants for energy or chitin synthesis. Chitin synthesis is a complex process requiring the coordination of multiple *chn* genes, the genes that may regulate symbiotic association (Lanfranco et al. 1999). Some researchers have also reported the role of involvement of genes encoding glyceraldehyde-3-phosphate dehydrogenase, β-tubulin and P-type ATPases in the AM fungus *Gigaspora rosea* (Harrier 2001). When plants obtain phosphate from the soil indirectly by mycorrhizal association transporters must take phosphate from the soil to the fungus before it is passed on to the plant. High-affinity phosphate transporters characterize fungi such as *Glomus versiforme*, *G. intraradices*, and *G. mosseae* and *P. indica*. Such transporters can be detected only in the extraradical mycelium and not in the fungal structures within the root (Buuren 1995). The exact mechanism by which phosphate is transferred from the fungus to the host plant, however, is still unclear (Buuren 1995). Recent research produced an X-ray crystal structure of a high affinity phosphate transporter, PiPT. This structure can provide information regarding phosphate affinity required for phosphate translocation. At the molecular level, signaling molecules are required for plant and fungi interaction (Pedersen et al. 2013).

The role of endogenous regulatory non-coding RNAs (microRNA) in many plant fungi symbiotic interactions has been studied. miRNAs, are involved in the production of plant hormones and in nutrient supply, regulation and signaling. For example, miRNA growth promotion promotes growth in *Oncidium* orchids with *P. indica* (Ye et al. 2014). Using uncolonized roots as a control, Ye et al. (2014) studied the involvement of miRNA in 8-week-old roots colonized by *P. indica*. They obtained conserved miroRNA as well as putative novel miRNAs from the colonized roots.

26.4 Use of Endophytes in the Mass Propagation of Orchids

Researchers have identified various uses of orchids, including ornamental medicinal and edible, and demand for them is high (Arditti 1967; Hossain 2009; Gutiérrez 2010; Pant 2013). Orchids are the first flowering plants ever commercially propagated by seeds and tissue culture (Arditti 1967; Pant 2013). Most of them are enlisted as threatened and endangered species. Thus, plant tissue culture, technique used to conserve endangered plant species as well as to mass produce high-quality, disease-free, genetically identical plants and their secondary metabolites, holds much promise, not just for conserving endangered species but for producing species in demand in large quantities.

Despite its advantages, however, plant tissue culture is significantly handicapped by the high mortality rate of *in vitro* plantlet when transferred to the field. Plants *in vitro* are fragile and weak immune systems, so they find it difficult to acclimatize and many die. To achieve the end of mass propagation, the use of endophytes as biological tools holds much promise. Endophytes overcome field problems such as the hardening of plant tissue and the acclimatization process in general, thereby resulting in higher survival rates of plantlets. Indeed, they have already been used to promote orchid seed germination and development and to promote plant growth in order to produce protective phytohormone (Zhang et al. 2013; Jian-wei et al. 2016).

Orchids are colonized by different species of endophytes at different stages of their lifecycles, but not all endophytes have a role in the growth and development. Those that are not called non-mycorrhizal endophytes though they may contribute to the production of plant hormones and secondary metabolites that are beneficial for plant growth and development. Generally, such endophytes are not host-specific (Sudheep and Sridhar 2012; Yuan et al. 2009). Both mycorrhizal and non-mycorrhizal endophytes have been used as a biological tool to stimulate the growth of *in vitro* plantlets and seedlings and to promote seed germination. Sour et al. (2015) reported the various endophytes present in eight different orchids, *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium hercoglossum*, *Dendrobium palpebrae*, *Torenia fournieri*, *Doritis pulcherrima*, *Dendrobium crumenatum*, *Dendrobium friedericksianum* and *Grammatophyllum specinoum*. Similarly, Wu et al. (2016) isolated endophytes from *D. officinale*, and they identified the antioxidant property of a novel compound.

The endophytes present in orchids are likely to be diverse, as the isolation of six endophytic fungi as three binucleated *Rhizoctonia* like fungi identified as *Tulasnella violea*, *Epulorhiza repen*, *Trichosporiella multisporum* along with *Beauvaria* and *Fusarium* from seven seed derived protocorms of *Dendrobium friedericksianum* (Khamchatra et al. 2016). The fact that Chen et al. (2013) isolated 961 endophytes, of which 217 belonged to the *Xyalariaceae* group of fungi were isolated from seven species of *Dendrobium*, *D. nobile*, *D. chrysotoxum*, *D. falconer*, and *D. aphyllum* among them, shows not just the diversity of endophytes but their specificity to the host as well. Similarly the diversity of endophytes was studied by Chen et al. (2012). About 127 endophytic were fungi isolated of which fungi belong

to *Rhizoctonia*-like strains and fungi belonging to order Cantharellales were identified from protocorm *D. nobile*. Whereas fungi belonging to Sebacinales were isolated from the root of *D. chrysanthum*.

These findings suggest that *in-situ* seed baiting is beneficial for screening for mycorrhizal fungi compatible with plant growth and therefore the mass propagation of epiphytic orchids. Already, a *Rhizoctonia*- like root endophyte was identified associated in the roots of *Dendrobium lancifolium* using morphological and nucleotide sequencing and phylogenetic analysis (Agustini 2016).

Zi et al. (2014) isolated two fungi, *Tulasnella* sp. and *Trichoderma* sp. from the seed baiting of *D. aphyllum*, *Tulasnella* sp. increased seed germination by 13.6%, protocorm formation by 85.7% and seedling development by 45.2%. In contrast, *Trichoderma* sp. suppressed seed germination by 26.4%. *Epulorhiza* isolated from *Cymbidium manni* showed seed germination by 6.5% and protocorm formation by 20.3%. Various types of fungi FDa17 (*Tulasnella* sp.), FDd1 (*Epulorhiza* sp.) and FCb4 (*Epulorhiza* sp.) were identified from *D. aphyllum*, *D. devianum* and *Cymbidium manni* respectively. FDa17 and FDd1 both promoted protocorm formation and seedling development of *D. aphyllum* and *D. devonianum* when they were inoculate separately. Over 60 days of growth, *D. devonianum* seeds inoculated only with FDa1 showed significant response: 44.36% of seeds grew to protocorms and 42.9% to seedlings. In contrast, only 14.46% of *D. aphyllum* seeds co-inoculated by FDa17 grew to protocorms and 12.07% to seedlings from the seed. Ding et al. (2014) reported the presence of *Rhizoctonia* sp. in *Liparis japonica* and found that they had a role in seed germination. Moreover, nitrogen fixation and the production of phytohormone such as salicylic acid (SA), indole-3-acetic acid (IAA), Zeatin and abscisic acid (ABA) were significantly improved by the endophytic bacteria *Sphingomonas paucimobilis* in orchid species (Yang et al. 2014a). These bacteria are potential to promote growth of *D. officinale*.

Many other endophytes appear to promote growth. Zhang et al. (2013) studied the unique mycorrihzal relationship of the F-23 fungus, *Mycena* sp. isolated from *Anoectochilus formosanus* Hayata. This fungus plays a role in the production of secondary metabolites such as kinsenosides and flavonoids, both of which ultimately help the growth and development of seedlings. Similarly, Dan et al. (2012a, b) isolated *Gliocladium*, *Epulrohiz*, *Fusarium, Moniliopsis, Cephalosporium* and *Mycena* species from *D.* nobile, *D. candidum* and A. *Roxburghii* also showed the role of those endophtyes in the growth and development of the orchid seedlings of those species. Hou and Guo (2009) focused more narrowly on the growth-promoting effect of the dark septate endophyte, *Leptodontidium* on seedlings of *D. nobile* and found the heights of shoots, number of new buds, number of roots, stem diameters and dry weights of fungal-colonized plantlets were all greater than those of uncolonized plantlets. Other efforts include those of Warcup (1981), who reported that endophytes such as *Sebacina vermifera*, *Tulasnella calospora*, *T. asymmetrica*, *T.cruciata, T. irregularis, T.violea* and *T. allantospora* are involved in seed germination in different orchid species (Table 26.1).

Table 26.1 List of endophytes that can be used as biological tool in the micropropagation of orchids

S.N.	Endophyte	Orchids	Role of endophytes	References
1.	*Tulasnella viole, Epulorhiza repe, Trichosporiella multisporum, Beauvari, Fusarium* sp.	*Dendrobium friedericksianum*	Seedling growth	Khamchatra et al. (2016)
2.	*Bacillus subtilis* BS87	*Anoectochilus Roxburghii, A. Formosanus*	Seedling growth	Jian-wei et al. (2016)
3.	*Rhizoctonia* sp.	*Dendrobium lancifolium*	Seed germination	Agustini (2016)
4.	*Tulasnella* sp., *Trichoderma* sp.	*D. aphyllum*	Seed germination	Zi et al. (2014)
5.	*Epulorhiza* sp.	*Cymibidium manni*	Seed germination	Zi et al. (2014)
6.	*Tulasnella* sp., *Epulorhiza* sp.	*D. aphyllum, D. devianum* and *Cymbidium manni*	Seedling growth	Zi et al. (2014)
7.	*Tulasnella*	*Dendrobium aphyllum*	Seed germination	Zi et al. (2014)
8.	*Sphingomonas paucimobilis*	*Dendrobium officinale*	Seedling growth	Yang et al. (2014a)
9.	*Rhizoctonia* sp.	*Liparis japonica*	Seed germination	Ding et al. (2014)
10.	*Xyalariaceae* sp.	*D. nobile, D. Chrysotoxum, D. falconer*, *D. aphyllum*	Seed germination	Chen et al. (2013)
11	*Piriformospora indica*	*Dactylorhiza majalis*	Seed germination	Varma et al. (2013)
12.	*Psathyrellaceae* sp.	*Coprinellus domesticus*	Seed germination	Yagame et al. (2013)
13.	*Cephalosporium* sp., *Epulrohiza* sp., *Gliocladium* sp., *Mycena dendrobii, Mycena anoectohila*	*D. nobile, D. candidum*	Seedling growth	Dan et al. (2012a)
14.	*Epulrohiza* sp., *Gliocladium* sp., *Mycena dendrobii, Mycena anoectohila, Moniliopsis* sp.	*Anoectohilus roxburghii*	Seedling growth	Dan et al. (2012b)
15	*Guignardia endophyllicola*	*Dendrobium crumenatum*	Seed germination	Mangunwardoyo (2012)

(continued)

Table 26.1 (continued)

S.N.	Endophyte	Orchids	Role of endophytes	References
16.	*Leptodontidium*	*Dendrobium nobile*	Seedling growth	Hou and Guo (2009)
17.	*Fusarium* sp., *Rhizoctonia* sp, *Rhizoctonia* sp., *Papulaspora* sp.	*Anacamptis pyramidalis, Ophrys fusca, Serapias vomeracea, Orchis sancta*	Seed germination	Gezgin and Eltem (2009)
18.	*Alternaria* sp., *Chaetomium* sp.	*Cymbidium eburneum*	Vegetative growth	Zhao and Liu (2008)
19.	*Fusarium* sp.	*Cymbidium eburneum*	Vegetative growth	Zhao and Liu (2008)
20.	*Fusarium* sp., *Trichoderma* sp., *Paeciloomyces* sp.	*Pleione yunnanensis*	Seed germination	Yang et al. (2008)
21.	*Mycena osmundicola*	*Gastrodia elata*	Seed germination	Kim et al. (2006)
22.	*Rhizoctonia* sp.	*Cymbidium goeringii*	Seedling growth	Wu et al. (2005)
23.	*Trichoderma* sp.	*Cymbidium goeringii*	Seedling growth	Huang (2004)
24.	*Mycena osmundicolor*	*Gastrodia elata*	Seed germination	Hong et al. (2002)
25.	*Sebacina* sp.	*Neottia nidus-avis*	Seed germination	Leake et al. (2002)
26.	*Phacodium* sp.	*Paphiopedilum armeniacum*	Seedling growth	Li (2001)
27.	*Fusarium* sp.	*Cypripedium reginae*	Seed germination	Vujanovic (2000)
28.	*Sebacina vermifera*	*Microtis unifolia*	Seed germination	Warcup (1981)
29.	*Tulasnella calospora*	*Diuris maculala, D. sulphurea, Orthocersa strictum, Spiranthes sinensis, T. Ixoides, T. Media, Dendrobium discolor, T. flexuosa, Calochilus* sp.	Seed germination	Warcup (1981)
30.	*T. asymmetrica*	*D. maculala, Orthocersa strictum, O. Strictum, S. Sinensis, T. Ixoides, T. Media, Dendrobium discolor, T. flexuosa, Calochilus* sp.	Seed germination	Warcup (1981)

(continued)

Table 26.1 (continued)

S.N.	Endophyte	Orchids	Role of endophytes	References
31.	*T. cruciata*	*D. maculala. Strictum, S. Sinensis, T. Ixoides, T. Media, Dendrobium discolor, T. flexuosa, Calochilus* sp.	Seed germination	Warcup (1981)
32.	*T. irregularis*	*O. strictum, D. maculala, S. Sinesis, T. Ixoides, T. Media, Dendrobium discolor, T. flexuosa endl, Calochilus* sp.	Seed germination	Warcup (1981)
33.	*T. violea*	*Thelymitra carnea, D. maculala Sm, O. Strictum, S. sinesis, T. media, Calochilus* sp.	Seed germination	Warcup (1981)
34.	*T. allantospora*	*Thelymitra cyanea, Diuris maculala, S. sinesis, Dendrobium discolor, T. flexuosa., Calochilus* sp.	Seed germination	Warcup (1981)

Over the years endophytes and mycorrhizal fungi have been isolated from plants in nature. Now, recent reports on the isolation and identification of endophtyes from host plants suggest that it is time to pursue this line of future investigation. Indeed, finding out more about microbe–plant interactions is crucial if we are to be able to conserve this remarkable creation of nature and find out more about how plants adapt to their native environments. As demand for orchids continues unabated and climate change creates havoc on ecosystems, we ignore this fact at great risk to ourselves and to the orchid itself.

Some of the endophytes isolated and identified from the roots of *Dendrobium moniliforme* in Central Department of Botany, Plant Biotechnology Laboratory. Tribhuvan University, Nepal is given in Fig. 26.4. (The data are unpublished; co-culture assay of orchid with isolated fungi to investigate their growth promoting effect is ongoing research in our laboratory).

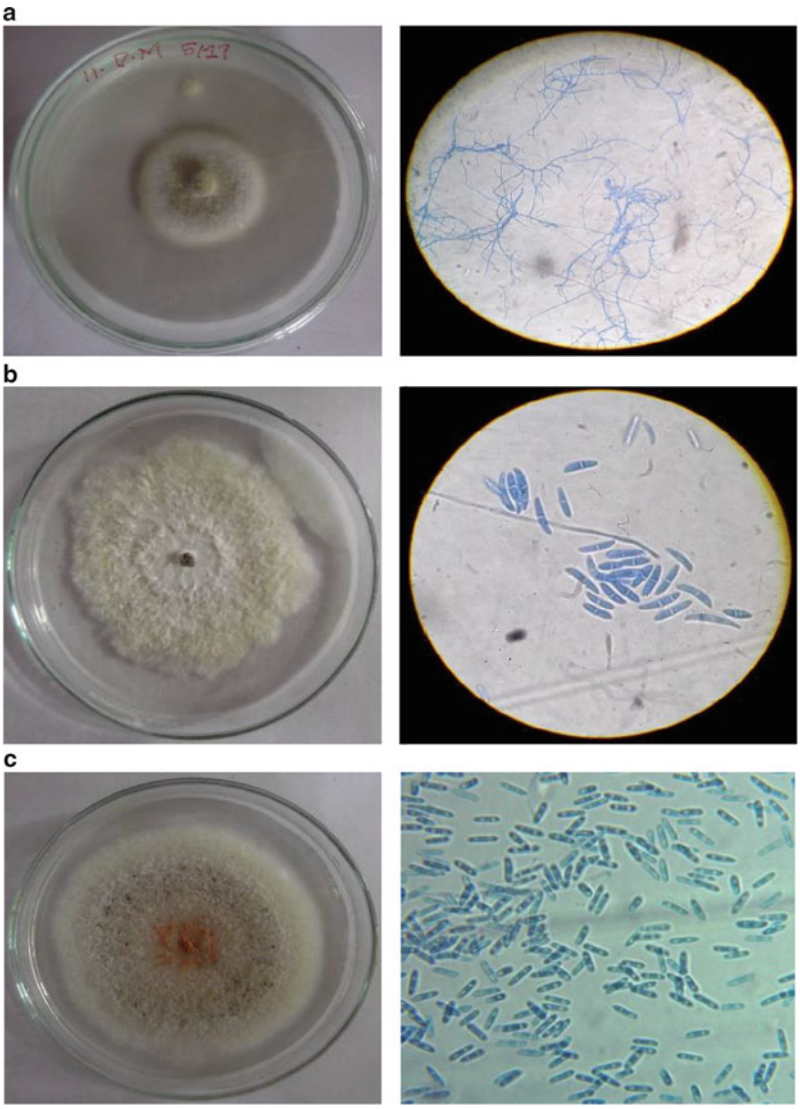


Fig. 26.4 (**a**) Bird's-eye view of a colony on PDA and a microscopic view at 40×. Seven-day-old *Leptosphaerulina* sp. Colony with a grayish-brown appearance Micro-morphology: Mycelium septate without sporulation. (**b**) Overse colony on PDA and microscopic at 40×. Seven-day-old *Fusarium oxyporum* colony with a white-to-pinkish floccose texture Micro-morphology: Microconidia with pointed and curved ends. (**c**) Overse colony on PDA and microscopic at 40×. Seven-day-old *Collectotrichum* colony with a sparse cottony-white periphery and an orange colored center. Micro-morphology: Conidia cylindrical-shaped with rounded ends

26.5 Conclusion

Fungi are essential for the growth and development of orchids and the production of secondary metabolites that are beneficial both for plants and human health. The fact that it is endophtyes that link microbes, plants and human makes such microorganisms a key topic of research. In addition, finding out more about microbes and plant interactions will benefit efforts to conserve orchids through mass propagation. Orchid sets an example for understanding the co-evolution of diverse form of microbes continuously interacting with a plant at different stages of lifecycle. Such research can be extended beyond orchids, too, to promote a wider understanding of the diverse forms of microbial interactions with plants.

References

Aggarwal S, Nirmala C, Beri S, Rastogi S, Adholeya A (2012) *In vitro* symbiotic seed germination and molecular characterization of associated endophytic fungi in a commercially important and endangered Indian orchid *Vanda coerulea* Griff. Ex Lindl. Eur J Environ Sci 2:33–42

Agustini V (2016) Short communication: Rhizoctonia-like fungi isolated from roots of *Dendrobium lancifolium* var. papuanum and *Calanthe triplicata* in Papua, Indonesia. Biodivers J Biol Divers 17:377–383

Alexander C, Hadley G (1985) Carbon movement between host and mycorrhizal endophyte during the development of the orchid *Goodyera repens* br. New Phytol 101:657–665

Al-Karaki G, Al-Raddad A (1997) Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7:83–88

Almeselmani M, Deshmukh PS, Sairam RK, Kushwaha SR, Singh TP (2006) Protective role of antioxidant enzymes under high temperature stress. Plant Sci 171:382–388

Arditti J (1967) Factors affecting the germination of orchid seeds. Bot Rev 33:1–97

Arditti J, Ernst R (1984) Physiology of orchid seed germination. In: Arditti J (ed) Orchid biology: reviews and perspectives. Cornell University Press, New York

Arditti J, Predgeon AM (1997) Orchid biology: reviews and prospectives, vol VII. Springer Science + Business Media, Dordrecht

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, koczowski 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

Baque A, Shin Y, Lee E, Paek K (2011) Effect of light quality, sucrose and coconut water concentration on the microporpagation of *Calanthe* hybrids ("Bukduseong" × "Hyesung" and "Chunkwang" × "Hyesung"). Aust J Crop Sci 5:1247–1254

Bonfante P, Perotto S (1995) Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol 130:3–21

Bulpitt CJ (2005) The uses and misuses of orchids in medicine. QJM Monthly JAPI 98:625–631

Buuren MJ (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Chand MB, Paudel MR, Pant B (2016) The antioxidant activity of selected wild orchids of Nepal. JCLM 4:731–736

Chapin FS III, Moilanen L, Kieland K (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361:150–153

Chen J, Wang H, Guo SX (2012) Isolation and identification of endophytic and mycorrhizal fungi from seeds and roots of *Dendrobium* (Orchidaceae). Mycorrhiza 22:297–307

Chen J, Zhang L, Xing Y, Wang Y, Xing X, Zhang D (2013) Diversity and taxonomy of endophytic *Xylariaceous* fungi from medicinal plants of *Dendrobium* (Orchidaceae). Plos One 8(3). https://doi.org/10.1371/journal.pone.0058268

Chen L, Zhang Q, Jia M, Ming Q, Yue W, Qin L (2016) Critical reviews in microbiology endophytic fungi with antitumor activities: their occurrence and anticancer compounds. Crit Rev Microbiol 42:454–473

Cribb PJ, Kell SP, Dixon KW, Barrett RL (2003) Orchid conservation: a global perspective. In: Dixon KW, Kell SP, Barrett RL, Cribb PJ (eds) Orchid conservation. Natural History Publications, Kota Kinabalu, Sabah, pp 1–2

Dan Y, Meng Z, Guo S (2012a) Effects of forty strains of orchidaceae mycorrhizal fungi on growth of protocorms and plantlets of *Dendrobium candidum* and *D. nobile*. Afr J Microbiol Res 6:34–39

Dan Y, Yu X, Guo S, Meng Z (2012b) Effects of forty-two strains of orchid mycorrhizal fungi on growth of plantlets of *Anoectochilus roxburghii*. Afr J Microbiol Res 6:1411–1416

Das J, Ramesh KV, Maithri U, Mutangana D, Suresh CK (2014) Response of aerobic rice to *Piriformospora indica*. IJEB 52:237–251

Deb CR (2008) Effects of different factorson immature embryoculture, PLBs differentiation and rapid mass multiplication of *Coelogyne suaveolens* (Lindl.) Hook. Int J Exp Biol 46:243

Dighe SM, Raval M, Shah AK (1986) Detection of nitrogen-fixing ability in an epiphytic orchid *Vanda testacea* (Linde) Reichb. F. Proc Natl Sci Acad Part B Biol Sci 52:515–518

Ding R, Chen XH, Zhang LJ, XD Y, Qu B, Duan R, Xu YF (2014) Identity and specificity of *Rhizoctonia*-like fungi from different populations of *Liparis japonica* (Orchidaceae) in northeast China. PLoS One 9(8)

Duponnois R, Garbaye J (1991) Mycorrhization helper bacteria associated with the Douglas fir-*Laccaria laccata* symbiosis: effects in aseptic and in glass house conditions. Ann For Sci 48:239–251

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

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Galdiano Junior RF, Nascimbem Pedrinho EA, Luque Castellane TC, de Macedo Lemos EG (2011) Auxin-producing bacteria isolated from the roots of *Cattleya walkeriana*, an endangered brazilian orchid, and their role in acclimatization. Rev Bras De Ciencia Do Solo 35:729–737

Gebauer G, Meyer M (2003) ^{15}N and ^{13}C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. New Phytol 160:209–223. https://doi.org/10.1046/j.1469-8137.2003.00872.x

Gennaro M, Gonthier P, Nicolotti G (2003) Fungal endophytic communities in healthy and declining *Quercus robur* L. and *Q. cerris* L. trees in Northern Italy. J Phytopathol 151:529–534

Gezgin Y, Eltem R (2009) Diversity of endophytic fungi from various Aegean and Mediterranean orchids (saleps). Turk J Bot 33:439–445

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari A, Johri A, Prasad R, Pereira E, Varma A, Tuteja N (2016) *Piriformospora indica*: Potential and significance in plant stress tolerance. Front Microbiol 7:332. https://doi.org/10.3389/fmicb.2016.00332

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, Giang PH, Kumari R, Prasad R, Sachdev M, Garg AP, Oelmuller 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, Heidelberg, pp 213–252

Gutiérrez R (2010) Orchids: a review of uses in traditional medicine, its phytochemistry and pharmacology. J Med Plants Res 4:592–638
Harrier L (2001) The arbuscular mycorrhizal symbiosis: a molecular review of the fungal dimension. J Exp Bot 52:469–478
He CN, Wang CL, Guo SX, Yang JS, Xiao PG (2006) A novel flavonoid glucoside from *Anoectochilus roxburghii* (Wall.) Lindl. J Integr Plant Biol 48:359–363
Hong IP, Kim H-K, Park J-S, Kim G-P, Lee MW, Guo SX (2002) Physiological characteristics of symbiotic fungi associated with the seed germination of *Gastrodia elata*. Mycobiology 30:22–26
Hossain MS (2009) Cost effective protocol for *in vitro* mass propagation of *Cybidium aloifolium* (L.) Sw. a medicinally important orchid. Eng Life Sci 9:444–453
Hou XQ, Guo SX (2009) Interaction between a dark septate endophytic isolate from *Dendrobium* sp. and roots of *D. nobile* seedlings. J Integr Plant Biol 51:374–381
Huang L (2004) Preliminary studies on mycorrhizal fungi in promoting the growth of orchid seedlings from tissue culture. Chin J Trop Crops 25:36–38
Jacquemyn H, Lenaerts M, Tyteca D, Lievens B (2013) Microbial diversity in the floral nectar of seven Epipactis (Orchidaceae) species. Microbiol Open 2:644–658
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. https://doi.org/10.5923/j.als.20160602.01
Khamchatra N, Dixon KW, Tantiwiwat S, Piapukiew J (2016) Symbiotic seed germination of an endangered epiphytic slipper orchid, *Paphiopedilum villosum* (Lindl.) Stein. from Thailand. S Afr J Bot 104:76–81
Kim Y, Jordan D, McDonald GA (1998) Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol Fertil Soils 26:79–87
Kim Y, Chang K, Ka K, Hur H, Hong I, Shim J, Lee M (2006) Seed germination of *Gastrodia elata* using symbiotic fungi, *Mycena osmundicola*. Mycobiology 34:79–82
Koide R (1992) Regulation of the vesicular-arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 43:557–581
Lanfranco L, Delpero M, Bonfante P (1999) Intrasporal variability of ribosomal sequences in the endomycorrhizal fungus *Gigaspora margarita*. Mol Ecol 8:37–45
Leake JR (1994) The biology of myco-heterotrophic ('saprophytic') plants. New Phytol 127:171–216
Leake JR, McKendrick SL, Bidartondo M, Read DJ (2002) Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. New Phytol 163:405–423
Li M (2001) Studies and applications on mycorrhiza of *Paphiopedilum armeniacum*. J Biol 18:17–18
Li AR, Guan KY, Stonor R, Smith SE, Smith FA (2013) Direct and indirect influences of arbuscular mycorrhizal fungi on phosphorus uptake by two root hemiparasitic *Pedicularis* species: do the fungal partners matter at low colonization levels? Ann Bot 112:1089–1098
Liu H, Luo Y, Liu H (2010) Studies of mycorrhizal fungi of chinese orchids and their role in Orchid conservation in China—a review. Bot Rev 76:241–262
Ma X, Kang J, Nontachaiyapoom S, Wen T, Hyde KD (2015) Non-mycorrhizal endophytic fungi from orchids. Curr Sci 109:72–80
Macdonald RM, Chandler MR (1981) Bacterium like organelles in the vesicular-arbuscular mycorrhizal fungus *Glomus caledonius*. New Phytol 89:241–246
Majumder PL, Bandyopadhyay S (2010) Stilbenoids and sesquiterpene derivatives of the orchids *Gastrochilum calcoelaria* and *Dendrobium amoenum*: application of 2D NMR spectroscopy in structural elucidation of complex natural products. J Indian Chem Soc 87:221–234
Majumder PL, Guha S, Sen S (1999) Bibenzyl derivatives from the orchid *Dendrobium amoenum*. Phytochemistry 52:1365–1369

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

Mandal SM, Chakraborty D, Dey S (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5:359–368. https://doi.org/10.4161/psb.5.4.10871

Mangunwardoyo W (2012) Frequency of endophytic fungi isolated from *Dendrobium crumenatum* Sw. (Pigeon orchid) and antimicrobial activity. Biodiversitas 13:34–39

Matsuoka H, Akiyama M, Kobayashi K, Yamaji K (2013) Fe and P solubilization under limiting conditions by bacteria isolated from carex kobomugi roots at the Hasaki coast. Curr Microbiol 66:314–321

Miller AJ, Cramer MD (2005) Root nitrogen acquisition and assimilation. Plant Soil 274:1–36

Moin S, Sahaya SB, Servin WP, Chitra DB (2012) Bioactive potential of *Coelogyne stricta* (D. Don) Schltr: an ornamental and medicinally important orchid. J Pharma Res 5:2191–2196

Moreira ASFP, Dos Santos Isaias RM (2008) Comparative anatomy of the absorption roots of terrestrial and epiphytic orchids. Braz Arch Biol Technol 51:83–93

Murashige T (1974) Plant propagation through tissue culture. Ann Rev Plant Physiol 25:135–166

Nicoletti R, Fiorentino A (2015) Plant bioactive metabolites and drugs produced by endophytic fungi of Spermatophyta. Agriculture 5:918–970

Pant B (2013) Medicinal orchids and their uses: tissue culture a potential alternative for conservation. Afr J Plant Sci 7:448–467

Pant B, Raskoti BB (2013) Medicinal orchids of Nepal. Himalayan Map House Pvt. Ltd., Nepal

Pant B, Thapa D (2012) In vitro mass propagation of an epiphytic orchid, *Dendrobium primulinum* Lindl. through shoot tip culture. Afr J Biotechnol 11:9970–9974

Pant B, Paudel M, Chand MB, Wagner SH (2016) Treasure troves of orchids in Central Nepal. Central Department of Botany, Nepal

Paudel MR, Chand MB, Karki N, Pant B (2015) Antioxidant activity and total phenolic and flavonoid contents of *Dendrobium amoenum* Wall ex Lindl. Botanica Orientalis. J Plant Sci:20–26

Pedersen BP, Kumar H, Waight AB, Risenmay AJ, Roe-Zurz Z, Chau BH, Stroud RM (2013) Crystal structure of a eukaryotic phosphate transporter. Nature 496:533–560

Pirozynski K (1989) Geological history of the *Glomaceae*, with particular reference to mycorrhizal symbiosis. Symbiosis 7:1–36

Pongener A, Deb CR (2011) In vitro regeneration of plantlets of *Cymbidium iridioides* D. Don using nodal segments as explants. Int J Appl Biotechnol Biochem 1:389–400

Pradhan S, Tiruwa B, Subedee BR, Pant B (2014) *In vitro* germination and propagation of threatened medicinal orchid, *Cymbidium aloifolium* (L.) Sw. through artificial seed. Asian Pac J Trop Biomed 4:971–976

Pradhan S, Regmi T, Ranjit M, Pant B (2016) Production of virus-free orchid *Cymbidium aloifolium* (L.) Sw. by various tissue culture techniques. Heliyon 2:e00176

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

Rasmussen HN, Rasmussen FN (2009) Orchid mycorrhiza: implications of a mycophagous life style. Oikos 118:334–345

Roy M, Yagame T, Yamato M, Iwase K, Heinz C, Faccio A, Selosse MA (2009) Ectomycorrhizal *Inocybe* species associate with the mycoheterotrophic orchid *Epipogium aphyllum* but not its asexual propagules. Ann Bot 104:595–610

Schachtman DP, Reid RJ, Ayling SM (1998) Update on phosphorus uptake phosphorus uptake by plants: from soil to cell. Plant Physiol 116:447–453

Scherer HW, Ahrens G (1996) Depletion of non-exchangeable NH_4-N in the soil-root interface in relation to clay mineral composition and plant species. Eur J Agron 5:1–7

Shahollari B, Varma A, Oelmüller R (2005) Expression of a receptor kinase in Arabidopsis roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton X-100 insoluble plasma membrane microdomains. J Plant Physiol 162:945–958

Sherameti I, Tripathi S, Varma A, Oelmüller R (2008) The root-colonizing endophyte *Pirifomospora indica* confers drought tolerance in *Arabidopsis* by stimulating the expression of drought stress-related genes in leaves. Mol Plant Microbe Interact MPMI 21:799–807

Shimura H, Matsuura M, Takada N, Koda Y (2007) An antifungal compound involved in symbiotic germination of *Cypripedium macranthos* var. rebunense (Orchidaceae). Phytochemistry 68:1442–1447

Sour V, Phonpho S, Soytong K (2015) Isolation of endophytic fungi from some orchid varieties. J Agric Technol 11:1243–1254

Stöckel M, Těšitelová T, Jersáková J, Bidartondo MI, Gebauer G (2014) Carbon and nitrogen gain during the growth of orchid seedlings in nature. New Phytol 202:606–615. https://doi.org/10.1111/nph.12688

Sudheep NM, Sridhar KR (2012) Non-mycorrhizal fungal endophytes in two orchids of Kaiga forest (Western Ghats), India. J For Res 23:453–460

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. J Plant Physiol 167:1009–1017

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. Plant Signal Behav 7:1621–1633

Tsavkelova EA, Cherdyntseva TA, Botina SG, Netrusov AI (2007) Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res 162:69–76

van Breusegem F, Dat JF (2006) Reactive oxygen species in plant cell death. Plant Physiol 141:384–390

Varma A, Verma S, Sudha, Sahay N, Bütehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl Environ Microbiol 65:2741–2744

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, Heidelberg, pp 231–254

Varma A, Fekete A, Srivastava A, Saxena AK, Frommberger M, Li D, Tripathi S (2013) *Piriformospora indica*. Soil Biol 33:201–219

Vij S (2002) Orchids and tissue culture: current status. Role of plant tissue culture in biodiversity conservation and economic development. Gyanodaya Prakashan, Nainital, p 491

Vujanovic V (2000) Viability testing of orchid seed and the promotion of colouration and germination. Ann Bot 86:79–86

Wang QX, Yan N, Ji DG, Li SY, Hu H (2013) *In vitro* growth and carbon utilization of the green-leaved orchid *Dendrobium officinale* are promoted by Mycorrhizal associations. Bot Stud 54:1

Warcup JH (1981) The Mycorrhizal relationships of Australian orchids. New Phytol 87:371–381

Wu J-R, Han S-F, Zhu Y-Y, Lu M, Wang G-P (2005) Ultrastructure of symbiosis mycorrhizal between *Cymbidium goeringii* and *Rhizoctonia* sp. J Nanjing For Univ Nat Sci Ed 29:105–108

Wu LS, Jia M, Chen L, Zhu B, Dong HX, Si JP, Han T (2016) Cytotoxic and antifungal constituents isolated from the metabolites of endophytic fungus DO14 from *Dendrobium officinale*. Molecules 21:1–14

Xu and Guo (1989) Fungus associated with nutrition of seed germination of *Gastrodia elata-Mycena osmundicola* Lange. Acta Mycologica Sinica 8:221–226

Yagame T, Funabiki E, Nagasawa E, Fukiharu T, Iwase K (2013) Identification and symbiotic ability of *Psathyrellaceae* fungi isolated from a photosynthetic orchid, *Cremastra appendiculata* (Orchidaceae). Am J Bot 100:1823–1830

Yang YL, Liu Z-Y, Zhu G-S (2008) Study on symbiotic seed germination of *Pleione bulbocodioide*s (Franch) Rolfe. Microbiology 35:909–912

Yang S, Zhang X, Cao Z, Zhao K, Wang S, Chen M, Hu X (2014a) Growth-promoting *Sphingomonas paucimobilis* ZJSH1 associated with *Dendrobium officinale* through phytohormone production and nitrogen fixation. Microbial Biotechnol 7:611–620

Yang Y, Zhao H, Barrero RA, Zhang B, Sun G, Wilson IW, Zhang Y (2014b) Genome sequencing and analysis of the paclitaxel-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431. BMC Genomics 15:69

Ye W, Shen CH, Lin Y, Chen PJ, Xu X, Oelmüller R, Lai Z (2014) Growth promotion-related miRNAs in *oncidium* orchid roots colonized by the endophytic fungus *Piriformospora indica*. PLos One 9(1). https://doi.org/10.1371/journal.pone.0084920

Yuan ZL, Chen YC, Yang Y (2009) Diverse non-mycorrhizal fungal endophytes inhabiting an epiphytic, medicinal orchid (*Dendrobium nobile*): estimation and characterization. World J Microbiol Biotechnol 25:295–303

Zeghad N, Merghem R (2013) Antioxidant and antibacterial activities of *Thymus vulgaris* L. Med Aromat Plant Res J 58:27–35

Zhang F, Lv Y, Zhao Y, Guo S (2013) Promoting role of an endophyte on the growth and contents of kinsenosides and flavonoids of *Anoectochilus formosanus* Hayata, a rare and threatened medicinal Orchidaceae plant. J Zhejiang Univ Sci B 14:785–792

Zhao JN, Liu HX (2008) Effects of fungal elicitors on the protocorm of *Cymbidium eburneum*. Ecol Sci 27:134–137

Zi XM, Sheng CL, Goodale UM, Shao SC, Gao JY (2014) In situ seed baiting to isolate germination-enhancing fungi for an epiphytic orchid, *Dendrobium aphyllum* (Orchidaceae). Mycorrhiza 24:487–499

Zimmer K, Hynson NA, Gebauer G, Allen EB, Allen MF, Read DJ (2007) Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyroloids and monotropoids (Ericaceae) and in orchids. New Phytol 175:166–175

Retraction Note to: Arbuscular Mycorrhizal Fungi: A Potential Tool for Restoration of Degraded Land

Razia Shuab, Rafiq Lone, Javaid Ahmad, and Zafar A. Reshi

Retraction Note to:
Chapter "Arbuscular Mycorrhizal Fungi: A Potential Tool for Restoration of Degraded Land" in: Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*, https://doi.org/10.1007/978-3-319-68867-1_22

The Editors have retracted this chapter by Shuab et al. [1] because it shows significant overlap with a previously published article by Asmelash et al. [2]. Razia Shuab, Rafiq Lone, Javaid Ahmad, and Zafar A. Reshi have not responded to any correspondence from the editor/publisher about this retraction.

[1] Shuab R., Lone R., Ahmad J., Reshi Z.A. (2017) Arbuscular Mycorrhizal Fungi: A Potential Tool for Restoration of Degraded Land. In: Varma A., Prasad R., Tuteja N. (eds) Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration. Springer, Cham

[2] Asmelash F, Bekele T and Birhane E (2016) The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands. Front. Microbiol. 7:1095. doi: 10.3389/fmicb.2016.01095

The retracted online version of this chapter can be found at https://doi.org/10.1007/978-3-319-68867-1_22

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1_27

Index

A. Varma et al. (eds.), *Mycorrhiza - Nutrient Uptake, Biocontrol, Ecorestoration*,
https://doi.org/10.1007/978-3-319-68867-1