Signaling and Communication in Plants

Christine M.F. Vos
Kemal Kazan *Editors*

Belowground Defence Strategies in Plants

Springer

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Series editor
František Baluška
Department of Plant Cell Biology, IZMB, University of Bonn, Kirschallee 1,
D-53115 Bonn, Germany

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Christine M.F. Vos • Kemal Kazan
Editors

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Editors
Christine M.F. Vos
CSIRO Agriculture Flagship
St Lucia, Queensland
Australia

Kemal Kazan
CSIRO Agriculture Flagship
St Lucia, Queensland
Australia

Scientia Terrae Research Institute
Sint-Katelijne-Waver, Belgium

ISSN 1867-9048 ISSN 1867-9056 (electronic)
Signaling and Communication in Plants
ISBN 978-3-319-42317-3 ISBN 978-3-319-42319-7 (eBook)
DOI 10.1007/978-3-319-42319-7

Library of Congress Control Number: 2016951305

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

Now that scientific focus is increasingly shifting to plant roots, it is a timely occasion to summarize our current knowledge on belowground defence strategies in plants by world-class scientists actively working in the area. The volume includes chapters covering belowground defence to main soil pathogens such as *Fusarium*, *Rhizoctonia*, *Verticillium*, *Phytophthora*, *Pythium*, and *Plasmodiophora*, as well as to migratory and sedentary plant parasitic nematodes. In addition, the role of root exudates in belowground plant defence is highlighted. Finally, accumulating evidence on how plants can differentiate beneficial soil microbes from the pathogenic ones is covered as well. Better understanding of belowground defences can lead to the development of environmentally friendly plant protection strategies effective against soilborne pathogens which cause substantial damage on many crop plants all over the world. The book will be a useful reference material for plant pathologists, agronomists, plant molecular biologists, as well as students working on these and related areas. The editors would like to thank all authors for their valuable contributions to this book.

St Lucia, Australia — Christine M.F. Vos
St Lucia, Australia — Kemal Kazan

Contents

Introduction to Belowground Defence Strategies in Plants

Christine M.F. Vos and Kemal Kazan

Abstract Plant roots have long been literally and figuratively hidden from sight, despite their unmistakable importance in a plant's life. Interactions between plant roots and soil microbes indeed seem to take place in a black box, but science is starting to shed some light into this box. This book aims to bring together our current knowledge on the belowground interactions of plant roots with both detrimental and beneficial microbes. This knowledge can form the basis for more environmentally friendly plant disease management of soil-borne pathogens and pests, and the book will be of interest to both plant scientists and students eager to discover the hidden part of a plant's daily life and survival.

Plants are multicellular photosynthetic organisms that have evolved from unicellular fresh water green algae. During their evolution, plants have acquired diverse capabilities that enabled them not only to survive but also to adapt and successfully colonize diverse land environments. In particular, the acquisition of roots or root-like structures that facilitate extracting water from soil rather than relying on limited amounts of moisture available on the soil surface has no doubt played an important role in plant's adaptation to life on land.

Obviously, roots are also essential for physical attachment of plants to the soil, as well as for nutrient uptake and interaction with soil biota. Plant roots continuously

C.M.F. Vos (✉)
Centre of Microbial and Plant Genetics, KU Leuven, Kasteelpark Arenberg 20, 3001 Leuven, Belgium

Department of Plant Systems Biology, Vlaams Instituut voor Biotechnologie (VIB), Technologie park 927, 9052 Ghent, Belgium

Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture, 306 Carmody Road, St Lucia, Brisbane, QLD 4067, Australia

Scientia Terrae Research Institute, Fortsesteenweg 30A, Sint-Katelijne-Waver, Belgium
e-mail: cvo@scientiaterrae.org

K. Kazan
CSIRO Agriculture St Lucia, 306 Carmody Road, St Lucia, QLD 4067, Australia

The Queensland Alliance for Agriculture & Food Innovation (QAAFI), Queensland Bioscience Precinct, University of Queensland, Brisbane, QLD 4072, Australia

C.M.F. Vos, K. Kazan (eds.), *Belowground Defence Strategies in Plants*, Signaling and Communication in Plants, DOI 10.1007/978-3-319-42319-7_1

explore the soil to sense and transmit diverse belowground signals needed to modify plant architecture. The interaction between plant roots and beneficial microbes (e.g., rhizobia or arbuscular mycorrhiza) can be highly advantageous for both parties and greatly contributes to agriculture. However, the belowground environment can be very hostile as well and plant roots are often threatened by various biotic and abiotic stress factors (e.g., lack of water, oxygen, nutrients; soil acidity, salinity, low temperatures, as well as pathogenic microbes). While the interaction between roots and nonpathogenic microbes can be beneficial, many pathogenic microbes and nematodes can inflict serious damage to roots, restricting plant growth, reducing yield, and even causing plant death. Therefore, plants must differentiate friends from foes to survive in a hostile environment, and the soil and plant roots play essential roles in this process.

Despite the importance of plant roots in the overall well-being of plants, crop breeding efforts aimed at improving biotic and abiotic stress tolerance have so far been mostly focused on the aboveground part of the plant. In fact, the roots are often referred to as "the hidden half," or the "black box," reflecting the neglected nature of plant root research. Similarly, although root pathogens cause enormous losses on our crop plants, root health has always been a difficult issue to deal with. Possible reasons for this are probably numerous but mainly include the complexity of the belowground environment.

Better understanding of the nature of the interaction between plant roots and both beneficial and pathogenic microbes can generate new knowledge leading to the development of novel strategies aimed at boosting plant productivity, while reducing crop losses. As Editors of this Springer book, our objective is to contribute to the ongoing efforts in this area by bringing together contributors who are leading researchers in their respective areas.

The first part of the book focuses on the general plant responses to soil microbes and the role that root exudates play in this process, both highly active research domains. The first chapter of this part (chapter "Belowground Defence Strategies in Plants: Parallels Between Root Responses to Beneficial and Detrimental Microbes") highlights the parallels that are increasingly emerging in plant root responses to beneficial and pathogenic microbes. The next chapter (chapter "Root Exudates as Integral Part of Belowground Plant Defence") details the essential and versatile roles of root exudates in belowground plant defences, impacting both detrimental and beneficial microbes.

The second part of the book then zooms in on the belowground defence strategies against specific root pathogens. Fungal root pathogens are represented by *Fusarium oxysporum* (chapter "Belowground Defence Strategies Against *Fusarium oxysporum*"), *Rhizoctonia* (chapter "Belowground Defence Strategies Against *Rhizoctonia*"), and *Verticillium* (chapter "Belowground Defence Strategies Against *Verticillium* Pathogens"). Next in line are the plant root responses to the oomycete pathogens *Phytophthora* (chapter "Belowground and Aboveground Strategies of Plant Resistance Against *Phytophthora* Species") and *Pythium* (chapter "Belowground Signaling and Defence in Host–*Pythium* Interactions"). Protists are represented by the clubroot pathogen *Plasmodiophora brassicae* (chapter

"Belowground Defence Strategies Against Clubroot (*Plasmodiophora brassicae*)"). Finally, nematodes are another detrimental soil pest with severe consequences for our worldwide food production. Chapter "Belowground Defence Strategies Against Sedentary Nematodes" covers sedentary nematodes, among which the highly damaging cyst and root-knot nematodes, while chapter "Belowground Defence Strategies Against Migratory Nematodes" deals with the migratory nematodes. The chapters in this part mainly focus on pathogen infection strategies and host resistance mechanisms, allowing an overview of the diverse nature of plant belowground defence strategies against pathogens and pests with varying lifestyles and infection strategies.

As already mentioned above, plants also seem to mount an initial defence response against beneficial microbes. Successfully colonizing microbes are able to overcome this and will assist the plant in its further belowground defences. This topic will be covered for the interactions between plant roots and the following beneficial microbes: nonpathogenic *Fusarium oxysporum* (chapter "Root Interactions with Nonpathogenic *Fusarium*"), *Trichoderma* (chapter "Belowground Defence Strategies in Plants: The Plant–*Trichoderma* Dialogue"), *Piriformospora indica* (chapter "Defence Reactions in Roots Elicited by Endofungal Bacteria of the Sebacinalean Symbiosis"), and arbuscular mycorrhizal fungi (chapter "Mitigating Abiotic Stresses in Crop Plants by Arbuscular Mycorrhizal Fungi"). The editors want to thank all authors for their valuable contributions, and wish you enjoyable reading of this book.

Part I
General Principles of Belowground Defence Strategies

Belowground Defence Strategies in Plants: Parallels Between Root Responses to Beneficial and Detrimental Microbes

Ruth Le Fevre and Sebastian Schornack

Abstract Plant roots, as underground structures, are hidden from view, difficult to work with and therefore typically understudied, especially in agricultural research. In addition to providing crucial support for aerial tissues and acquiring nutrients, roots engage with filamentous microorganisms in the soil. These interactions have outcomes ranging from positive to negative and therefore roots must respond appropriately to different microbes to ensure plant survival. While leaf responses to filamentous pathogens have been well researched, we lack comparative information from roots. Moreover, we lack knowledge on the extent of overlap of root responses to microbes that share similarities in morphology, biochemistry and colonisation strategy but that result in different outcomes. In this chapter, we highlight current knowledge on parallels in root responses to beneficial and detrimental filamentous microorganisms. We also emphasise the importance of root studies and advocate the development of new host systems that allow comparative root–microbe interaction research. Ultimately, understanding of this field at the molecular level could inform breeding for pathogen resistance in crops while promoting cooperative root interactions with other microbes.

1 Introduction

Plant roots are in constant contact with microorganisms in the soil. Interactions with specific microbes can lead to beneficial or detrimental outcomes for plants and significantly affect plant growth and development. Therefore, distinguishing between a potential mutualist and pathogen and responding appropriately are paramount to plant survival because pathogenic microorganisms can destroy plant tissue, while beneficial microorganisms can aid nutrient uptake and confer resistance to biotic and abiotic stresses.

R. Le Fevre • S. Schornack (✉)
Sainsbury Laboratory (SLCU), University of Cambridge, Cambridge, UK
e-mail: sebastian.schornack@slcu.cam.ac.uk

C.M.F. Vos, K. Kazan (eds.), *Belowground Defence Strategies in Plants*, Signaling and Communication in Plants, DOI 10.1007/978-3-319-42319-7_2

In leaves, responses to and interactions with pathogens have been well characterised. In roots, pathogen studies are fewer; however, beneficial interactions are well studied. Interestingly, the morphologies and mechanisms of colonisation of plant roots by filamentous microbes that have different effects on plants are similar. Therefore there is likely to be significant overlap in root responses to these different microbes. However, our research into the extent of this overlap is hampered, partly because suitable systems for comparative studies between these different interactions are rare (Rey and Schornack 2013). A greater understanding of microbial interactions with plant roots could enable new ways of protecting crops from those that are detrimental while promoting those that are beneficial. This is especially important considering future agricultural settings where we may rely on beneficial plant–microbe interactions, for enhancing plant nutrition when fertilizers become limited, and simultaneously aim to reduce disease in crops in order to maximise yield.

In this chapter we review recent work that highlights what is known about root responses to beneficial and detrimental filamentous microbes. We highlight the importance of studies in roots and advocate the development of new host systems, both plant and microorganism, which allow comparative root–microbe interaction studies.

2 The Study of Root–Microbe Interactions

The interactions of soil microbes with plant roots are typically understudied, especially in agricultural research, because as underground structures they are hidden from view and difficult to work with (Balmer and Mauch-Mani 2013). However, given the absolute importance of roots for nutrient and water uptake, anchoring and support of aerial tissue and direct interaction with the soil environment and microbiome, it is critical we understand more about these plant tissues and the associations they form with microorganisms. Understanding and engineering root–microbe interactions will help us find possible strategies to improve crop yield, stress resilience and pathogen protection.

Above- and belowground plant tissues are exposed to different microorganisms. The soil environment contains millions of filamentous microbes (fungal and other eukaryotic microorganisms with fungal morphologies, such as oomycetes) that are in constant proximity to or contact with plant roots (van der Heijden et al. 2008). Therefore, it is reasonable to hypothesise that recognition of and downstream responses to microbes in shoots and roots will differ (Balmer and Mauch-Mani 2013). Appropriate and timely responses in roots are especially important so as not to be constitutively activated, as this could impose fitness costs (De Coninck et al. 2015). Schreiber et al. (2011) demonstrated that the roots, but not leaves, of *Arabidopsis thaliana* were susceptible to the pathogenic fungus *Magnaporthe oryzae*, indicating that the defence situation below and above ground to this microbe is indeed different. However, the use of mutants has illustrated that plant

defence signalling pathways are generally conserved between above- and belowground tissues (De Coninck et al. 2015). As most work on plant responses to pathogenic microbes has been done in aboveground tissue, we can use our knowledge from leaves to test root responses to pathogens and highlight common and contrasting principles.

Microbes engage in a range of interactions with plant roots. Beneficial symbioses facilitate plant nutrient uptake and can increase abiotic and biotic stress tolerance. Detrimental pathogenic interactions result in nutrient loss and disease. We know most about the associations at the more extreme ends of the spectrum (Fig. 1b). However, what are less well understood are the intermediate interactions, such as those with endophytes (Jumpponen and Trappe 1998; Franken 2012). Filamentous endophytic fungi (such as the dark septate endophytes, DSE) persist in plant roots seemingly without causing disease, but the outcomes, in terms of effects on the plant, can vary from negative to neutral to positive depending on the specific microbe–host combination (Jumpponen 2001). Given that the microbe and the host environment can influence the outcome of an interaction, comparative studies that keep one interaction partner constant (one microbe in multiple hosts or multiple microbes with similar lifestyles within one host) would allow characterisation of the contribution of each partner. Additionally, appropriate plant host and microbial systems (see Table 2) to study these associations could help to answer many interesting questions arising from the topic of root–microbe interactions:

- Why do some microbes have different lifestyles on different plant tissues? (Sect. 2.2.1)
- How and why do some microbes engage in different interactions with different hosts? (Sect. 3.5)
- Are plant defence responses activated and suppressed in a microbe-specific or lifestyle-specific manner? (Sects. 4.1–4.3)
- Are structures formed by beneficial and detrimental microbes analogous? (Sects. 4.2 and 4.3, Fig. 1)
- Do plant traits similarly or differentially affect filamentous microbes with different lifestyles in roots? (see Table 1)

Understanding how the outcomes of plant root–microbe interactions are controlled would ultimately provide inroads to promote beneficial partnerships while suppressing detrimental ones.

2.1 Plant Systems

To better understand root responses to different microbes, a variety of appropriate plant and microbial systems to work with are needed. Studying root responses to different microbes that engage in a range of interactions in the same plant species would be advantageous.

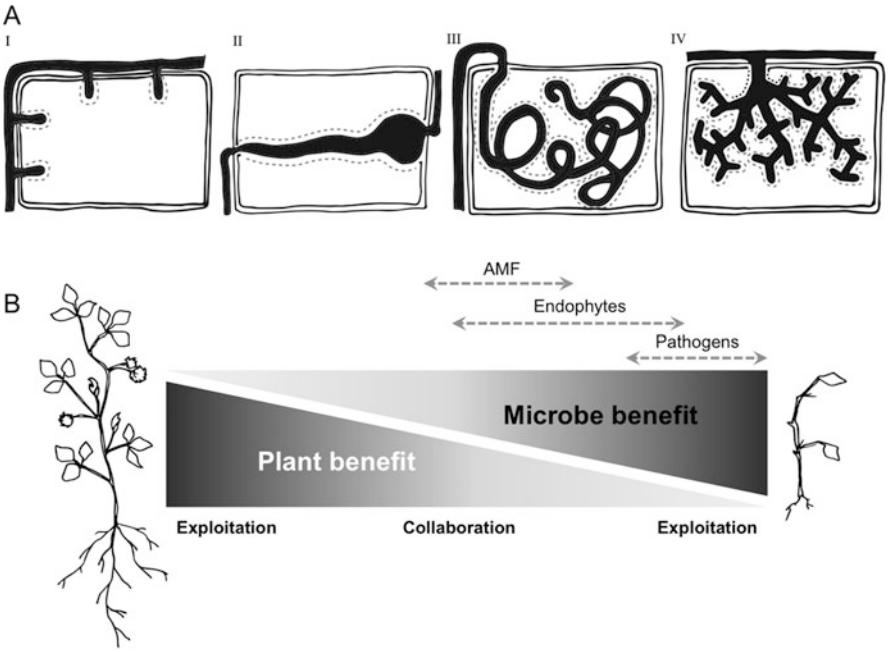

Fig. 1 Microbes engage in a spectrum of interactions with plant roots. (**a**) During root colonisation microbes form a variety of intracellular structures that can facilitate nutrient transfer, effector delivery to modulate host immune responses or simply the progress of growth through root cells. Although the microbe penetrates the cell wall (*outer solid line*), the protoplast remains intact and, at least in the case of **I**, haustoria, and **IV**, arbuscules, a modified membrane (*dashed line*) that contains a distinct protein complement from the rest of the plasma membrane (*inner solid line*) encases the microbial structure. *M. oryzae* transverses root cells as in **II** and *P. indica* forms coils insides cells as in **III**, but nothing is known about the membranes that surround these structures and whether they are also different from the plasma membrane as in **I** and **II**. (**b**) Root–microbe interactions lie on a spectrum and cannot be compartmentalised into beneficial or detrimental without taking into consideration the interaction in context of environmental factors and host/microbe genotype. This spectrum has been described elsewhere as the mutualism–parasitism continuum (Mandyam and Jumpponen 2015). *Dashed arrows* for arbuscular mycorrhizal fungi (AMF), endophytes and pathogens represent perceived extents to which microbe and plant benefit from the interactions they engage in

Medicago truncatula has been used extensively for symbiosis research and has been instrumental for identifying genes affecting interactions with beneficial arbuscular mycorrhizal fungi (AM fungi, Table 1, Ane et al. 2008). With this resource we are now able to determine whether these same genes are important for colonisation of roots by other microbes, including pathogens (Table 1, Wang et al. 2012; Gobbato et al. 2012, 2013; Rey et al. 2013, 2015).

Given that the three most important food crops (maize, wheat and rice) are monocots, with root architectures divergent from dicots, the use of monocot plants is also important for monocot versus dicot root response comparisons. In this regard rice and maize are good candidates as plant systems for root–microbe interactions as they have been used for AM fungi and pathogen research (see Table 2).

Table 1 Examples of plant genes implicated in colonisation of roots by beneficial and detrimental filamentous microbes

Gene	Plant species	Protein	Colonisation phenotype upon gene mutation or knock-down		References
			Beneficial	Detrimental	
HvMLO	*Hordeum vulgare*	Plasma membrane-localised seven trans-membrane domain protein	Reduced colonisation by *Funneliformis mosseae*	Unknown	Ruiz-Lozano et al. (1999)
MtDMI1/ *LjCASTOR* + *LjPOLLUX*	*Medicago truncatula*, *Lotus japonicus*	K^+ ion channel	Myc–	Increased resistance to *Verticillium albo-atrum*	Catoira et al. (2000); Ane et al. (2004); Imaizumi-Anraku et al. (2005); Charpentier et al. (2008); Ben et al. (2013)
MtDMI2/ *MtNORK*/ *LjSymRK*	*M. truncatula*, *Medicago sativa*, *L. japonicus*	MAL-LRR-RLK	Myc–	No alteration in *Phytophthora palmivora* colonisation	Wegel et al. (1998); Catoira et al. (2000); Endre et al. (2002); Rey et al. (2015); Stracke et al. (2002)
MtDMI3/ *LjCCamK*	*M. truncatula*, *L. japonicus*	CCaMK	Myc–, no cytoplasmic aggregations under *Gigaspora gigantea*	No alteration in *Colletotrichum trifolii* colonisation but altered in cytoplasmic aggregation under hyphopodia or from contact with *Phoma medicaginis*	Genre et al. (2009); Levy et al. (2004); Morandi et al. (2005)
MtLIN/ *LjCERBERUS*	*M. truncatula*	E3 ligase	Reduced colonisation by *Rhizophagus irregularis* and *Gigaspora margarita*. Infection structures normal but defective in hyphal elongation	More susceptible to *P. palmivora*	Rey et al. (2015); Takeda et al. (2013)

(continued)

Table 1 (continued)

Gene	Plant species	Protein	Colonisation phenotype upon gene mutation or knock-down		References
			Beneficial	Detrimental	
MtLYK3/ *LjNFR1*	*M. truncatula*, *L. japonicus*	LysM-RLK	Reduced myc colonisation in both *nfr* and *hcl* mutants (phenotype was stronger for *nfr* than *hcl*)	*hcl2* but not *hcl1* is more susceptible to *P. palmivora*	Rey et al. (2015); Zhang et al. (2015)
MtNFP/ *LjNFR5*	*M. truncatula*, *L. japonicus*	LysM-RLK	Myc+ but involved in MYC-signal elicited root branching stimulation	More susceptible to *A. euteiches*, *C. trifolii*, *V. albo-atrum* and *P. palmivora*	Achatz et al. (2010); Ben et al. (2013); Maillet et al. (2011); Rey et al. (2015)
MtNSP1/ *LjNSP1*	*M. truncatula*, *L. japonicus*	GRAS TF	Reduced colonisation and infection frequency by *R. irregularis*	Increased resistance to *V. albo-atrum*	Ben et al. (2013); Liu et al. (2011); Takeda et al. (2013)
MtNSP2	*M. truncatula*	GRAS TF	Reduced colonisation by *R. irregularis*, reduced MYC-signal elicited root branching stimulation	No alteration in *P. palmivora* colonisation	Maillet et al. (2011); Rey et al. (2015)
MtRAM1	*M. truncatula*	GRAS TF	Reduced colonisation by *R. irregularis*, suppressed MYC-signal elicited root branching stimulation	No alteration in *Aphanomyces euteiches* or *P. palmivora* colonisation	Gobbato et al. (2012); Maillet et al. (2011); Rey et al. (2015)
MtRAM2	*M. truncatula*	GPAT	Reduced myc colonisation by *R. irregularis* and *Glomus hoi*	Reduced colonisation by *P. palmivora* and *A. euteiches*	Gobbato et al. (2013); Wang et al. (2012)
MtROP9	*M. truncatula*	G-protein	*R. irregularis* colonisation promoted in *MtROP9* RNAi plants	*A. euteiches* colonisation promoted in *MtROP9* RNAi plants	Kiirika et al. (2012)
MtVPY	*M. truncatula*	Protein with N-terminal major sperm protein domain	Myc+ but *R. irregularis* produces deformed hyphopodia, more intraradical hyphae and no arbuscules	Unknown	Murray et al. (2011); Pumplin et al. (2010)

OsCERK1	*Oryzae sativa*	LysM-RLK	Reduced *R. irregularis* colonisation in *cerk1* rice	Increased *Magnaporthe oryzae* susceptibility (in leaves but roots not yet tested)	Miyata et al. (2014); Kishimoto et al. (2010); Mentlak et al. (2012); Zhang et al. (2015)
OsCEBiP	*O. sativa*	LysM chitin-binding protein	No alteration in myc phenotype in *cebip* rice	Increased *M. oryzae* susceptibility (in leaves but roots not yet tested)	Kaku et al. (2006); Kishimoto et al. (2010); Kouzai et al. (2014); Mentlak et al. (2012); Miyata et al. (2014)

Table 2 Examples of filamentous microorganisms that permit comparative studies of detrimental and beneficial interactions in roots

Microbe species	Taxonomic group	Perceived root interaction	Plant hosts	Penetration of root surface	Intracellular interface	References
Bipolaris sorokiniana	Fungus—Ascomycete	Detrimental	Small grain cereals	Appressoria	Intracellular hyphae	Kumar et al. (2002); Carlson et al. (1991)
Colletotrichum spp. (*Colletotrichum graminicola* and *Colletotrichum trifolii*)	Fungus—Ascomycete	Detrimental after greatly extended biotrophy	Maize, *Medicago truncatula*	Intercellular, melanised appressoria and hyphopodia	Intracellular hyphae	Genre et al. (2009); Sukno et al. (2008); Venard and Vaillancourt (2007)
Dark septate endophytes (e.g. *Phialocephala fortinii* and *Chloridium paucisporum*)	Fungi—Ascomycetes	Detrimental to beneficial	A huge range, including *Arabidopsis thaliana* and leek	Intracellular (root hairs) and intercellular	Intracellular hyphae	Mandyam and Jumpponen (2015)
Ericoid mycorrhiza (e.g. *Rhizoscyphus ericae*)	Fungi—Ascomycetes	Neutral to beneficial	Ericaceous plants and liverworts, e.g. *Pachyschistochila splachnophylla*	Intracellular (through rhizoids in liverworts)	Intracellular coils	Pressel et al. (2008)
Fusarium spp.	Fungus—Ascomycete	Detrimental (hemibiotrophic)	Many, including wheat and barley, *A. thaliana* and *Brachypodium distachyon*	Intercellular and intracellular	Intracellular hyphae	Lyons et al. (2015); Peraldi et al. (2011); Scherm et al. (2013)
Magnaporthe oryzae	Fungus—Ascomycete	Detrimental after greatly extended biotrophy	Rice, barley, *A. thaliana*	Hyphopodia, intracellular	Intracellular hyphae	Marcel et al. (2010); Sesma and Osbourn (2004); Schreiber et al. (2011)

Trichoderma spp.	Fungus—Ascomycete	Neutral to beneficial. Some cell death	Many, including maize, wheat and tomato	Intracellular, through root hairs/rhizodermis	None	Moran-Diez et al. (2015); Shukla et al. (2015); Yedidia et al. (1999)
Verticillium spp. (*Verticillium longisporum* and *Verticillium albo-atrum*)	Fungus—Ascomycete	Detrimental	Many, including *M. truncatula*, *A. thaliana*, alfalfa, tomato and Brassica oilseed crops	Intercellular and intracellular	Intracellular hyphae	Ben et al. (2013); Johansson et al. (2006)
Laccaria bicolor (Ecomycorrhiza)	Fungus—Basidiomycete	Beneficial	Trees, including *Populus* spp. such as black cottonwood (*Populus trichocarpa*)	Intercellular	None	Tschaplinski et al. (2014)
Piriformospora indica	Fungus—Basidiomycete	Neutral to beneficial. Some cell death	Many, including *A. thaliana*, barley and maize	Intercellular	Intracellular hyphae (coils)	Lahrmann et al. (2013); Kumar et al. (2009)
Rhizoctonia solani	Fungus—Basidiomycete	Detrimental	Many, including wheat, *B. distachyon*, rice, potato, maize, sugar beet, bean, lupin, cotton, lettuce, melon, *M. truncatula*	Wounds, intercellular	None	Schneebeli et al. (2015); Anderson et al. (2013); Garcia et al. (2006)
Ustilago maydis	Fungus—Basidiomycete	Neutral	Maize, *M. truncatula*	Intercellular	Intracellular hyphae	Mazaheri-Naeini et al. (2015)
Glomeromycota arbuscular mycorrhizal fungi (*e.g. Rhizophagus irregularis*)	Fungus—Glomeromycete	Beneficial (potentially detrimental on non-mycorrhizal hosts such as *A. thaliana*)	A huge range, including *M. truncatula*, rice, *A. thaliana* and liverworts (although not the model system *Marchantia polymorpha* var. *vulgaris*)	Hyphopodia	Arbuscules	Bonfante and Genre (2008); Ligrone et al. (2007); Parniske (2008); Russell and Bulman (2005); Veiga et al. (2013); Ligrone et al. (2007); Veiga et al. (2013); Wang and Qiu (2006)

(continued)

Table 2 (continued)

Microbe species	Taxonomic group	Perceived root interaction	Plant hosts	Penetration of root surface	Intracellular interface	References
Endogone fungi (Mucoromycotina)	Fungi—Zygomycete	Neutral to beneficial	Basal and higher land plants such as *Marchantia*, tobacco and tomato	Unknown	Intracellular hyphal coils and lumps	Daft and Nicolson (1966, 1969); Field et al. (2015); Russell and Bulman (2005)
Aphanomyces euteiches	Oomycete—Heterokontophyte	Detrimental	*M. truncatula*, pea and other legumes	Intracellular and intercellular	Haustoria reported once	Djebali et al. (2009, 2011); Gaulin et al. (2007); Franken et al. (2007)
Phytophthora spp. (e.g. *P. palmivora*, *P. sojae*)	Oomycete—Heterokontophyte	Detrimental	Many (species dependent) including soybean, lupin, *M. truncatula*	Intracellular, appressoria	Haustoria	Rey et al. (2015); Drenth and Guest (2004); Kroon et al. (2012)
Pythium spp.	Oomycete—Heterokontophyte	Detrimental	Many, including wheat and soybean	Intracellular, swollen hyphae	Intracellular hyphae	Kageyama (2014); Van Buyten and Hofte (2013)
Plasmodiophora brassicae	Rhizaria—Cercozoa	Detrimental	Brassicaceae	Through root hairs	Intracellular plasmodia	Gludovacz et al. (2014); Kageyama and Asano (2009)

Importantly, recent work in rice has shown that there are root type-specific transcriptional responses to colonisation by AM fungi (Gutjahr et al. 2015). This highlights the need for root type-specific microbe interactions to be studied independently.

Barley and wheat are other suitable monocot candidate systems of significant economic relevance. Work in crops is especially advantageous because it negates the need for knowledge transfer from model plant species. Both barley and wheat engage in beneficial symbiotic interactions with AM fungi and are affected by *Fusarium*, *Rhizoctonia* and *Pythium* root pathogens. Additionally the barley–*Piriformospora indica* (a model endophytic fungus) root interaction is already an established research system (Table 2).

Arabidopsis has been used to investigate *P. indica*, *M. oryzae*, *Verticillium* and *Fusarium*–root interactions. While it is a non-mycorrhizal species, it may still undergo interactions with these fungi (Veiga et al. 2013). Other advantages of using *Arabidopsis* as a model include the accessibility of mutants and extent of genome resources and its convenience in size and life cycle.

Ultimately, the use of a range of monocot and dicot model plant species will help to uncover core microbial accommodation programmes and those that are host species specific for microbes with specific lifestyles. The evolutionary conservation of these programmes can also be studied as lower descent plants, such as liverworts and hornworts, are also colonised by AM fungi and other filamentous microbes (see Table 2, Russell and Bulman 2005; Bonfante and Genre 2008).

2.2 Microbial Systems

In the following sections, we introduce additional microbial systems that are particularly suited for comparative studies between root responses to pathogens and mutualists.

2.2.1 Foliar Fungal Pathogens

The study of fungal pathogens and responses to pathogen colonisation in roots has been neglected in comparison to leaves, but this is not for a lack of root pathogens (see, e.g. *Fusarium* in chapter "Belowground Defence Strategies Against *Fusarium oxysporum*", *Rhizoctonia* in chapter "Belowground Defence Strategies Against *Rhizoctonia*", *Verticillium* in chapter "Belowground Defence Strategies Against *Verticillium* Pathogens" and *Pythium* in chapter "Belowground Signalling and Defence in Host–*Pythium* Interactions" in this book). Other non-pathogenic root-infecting fungi have also been introduced elsewhere (*Trichoderma* in chapter "Belowground Defence Strategies in Plants: The Plant–*Trichoderma* Dialogue", *P. indica* in chapter "Defence Reactions in Roots Elicited by Endofungal Bacteria of the Sebacinalean Symbiosis" and AM fungi in chapter "Mitigating Abiotic

Stresses in Crop Plants by Arbuscular Mycorrhizal Fungi"). Interesting, there is accumulating evidence that many foliar pathogens, including the rice blast fungus *M. oryzae*, anthracnose causing hemibiotrophic (i.e. exhibiting both symptomless biotrophic growth and tissue destroying necrotrophic life stages) *Colletotrichum* spp. and smut fungus *Ustilago maydis*, are also able to infect roots—although knowledge on their occurrence as natural root pathogens is often limited (Table 2, Dufresne and Osbourn 2001; Sukno et al. 2008; Mazaheri-Naeini et al. 2015). There is, therefore, the potential to use foliar fungal pathogens to facilitate the study of root–microbe interactions. Their classification as disease-causing pathogens, however, may have to be revisited in the root situation, as their associations with underground plant tissues appear less aggressively parasitic and more endophytic. Interestingly, penetration structures formed by some leaf pathogens on roots appear more similar structurally to those produced by AM fungi (see Sect. 4.2). Additionally, inside root tissue, *M. oryzae*, *Colletotrichum graminicola* and *U. maydis* engage in intercellular and intracellular biotrophic growth, and symptoms of disease are either extremely delayed, as for *M. oryzae* and *C. graminicola*, or do not seem to occur at all, as for *U. maydis* (Sukno et al. 2008; Marcel et al. 2010; Mazaheri-Naeini et al. 2015). In this way, these aggressive foliar pathogens appear to have different programmes for colonisation of different plant tissues and become more endophytic in lifestyle when infecting plant roots. One hypothesis for this is an absence of strong immune response signalling in some root tissues (such as the cortex) compared to leaves, enabling an extended period of biotrophic growth, although this has yet to be tested. As an avenue for future research, it will be especially interesting to discover just how many leaf pathogens also engage in root colonisation.

2.2.2 Oomycete Pathogens

Oomycetes are root- and shoot-infecting fungus-lookalikes which are taxonomically unrelated to fungi and differ from them in some structural and lifestyle features (Fawke et al. 2015). *Aphanomyces euteiches* and *Phytophthora palmivora* are root rot-causing oomycete pathogens. While *A. euteiches* infects legumes, *P. palmivora* has a very broad host range and infects many monocot and dicot species (Drenth and Guest 2004; Agrios 2005). *P. palmivora* is particularly interesting as it forms specialised intracellular lateral hyphal branches, termed haustoria, inside root cells (Rey et al. 2015). *A. euteiches* may also form haustoria, although so far they have only been reported from a single study (Franken et al. 2007). Haustoria have been best studied as structures formed by biotrophic and hemibiotrophic pathogens that cause foliar diseases, and parallels have been drawn between these structures and the intracellular branched hyphal arbuscules formed by AM fungi (chapter "Mitigating Abiotic Stresses in Crop Plants by Arbuscular Mycorrhizal Fungi", Sect. 4.3, Rey and Schornack 2013). Also, specialised plant-derived membranes form around haustoria as they do for AM fungi (see Sect. 4.3.2). Therefore, in comparison with AM fungi, we can use

P. palmivora to increase our understanding of the formation and function of intracellular microbial structures and interfaces.

3 Can I Stay or Must I Go? Parallels in Root Responses to Beneficial and Detrimental Microbes at the Tissue Level

In the interaction of plant roots with filamentous microbes, complex two-way signalling occurs between host and potential invader. Depending on the microbe, root responses can facilitate long-term accommodation and mutualistic associations or act defensively to try and rid plant tissue of the foreign body. Parallels in root responses to microbes with different lifestyles occur at the molecular level (Sect. 4) and also at the tissue level as discussed in the following sections.

3.1 Nutrition Status

The nutrient status of the soil affects root responses to potential microbial interactions. For example, if sufficient, accessible phosphate is present in the soil, it is directly acquired through the roots. As a result, colonisation by AM fungi and the symbiotic-phosphate uptake pathway are suppressed. Additionally, production of strigolactone (SL) phytohormones by plant roots, which stimulate germination of AM fungal spores and hyphal branching, is reduced if phosphate levels are non-limiting (Gu et al. 2011). Conversely, if phosphate and nitrate levels are limiting, roots respond by producing and secreting increased amounts of SL (Yoneyama et al. 2007, 2013). Mutant plants defective in SL production, *nsp1* and *nsp2* (genes that control SL biosynthesis), are compromised in colonisation by AM fungi compared to wild-type plants (Liu et al. 2011; Lauressergues et al. 2012; Takeda et al. 2013; Delaux et al. 2013). Interestingly, SL-deficient *nsp1* mutant *Medicago* plants were more resistant to the pathogenic microbe *Verticillium albo-atrum* than the wild type (Table 1, Ben et al. 2013). Production of SL by roots in response to nutrient status is therefore important for colonisation by beneficial microbes and may also affect colonisation by detrimental microbes, although the effects of SLs on growth and branching of filamentous microbes other than AM fungi are unclear. When the effects of the synthetic strigolactone GR24 were tested on *P. indica* and the root pathogen *Fusarium oxysporum* f. sp. *lycopersici*, no effect in growth or branching was reported (Steinkellner et al. 2007; Steinkellner and Mammerler 2007). However, in another study, GR24 actually inhibited radial growth of *F. oxysporum* and *Fusarium solani* and increased the number of branches in the former, but not the latter microbe (Dor et al. 2011).

3.2 Root System Morphology and Root Branching

Responses to mutualistic and parasitic interactions result in various changes to root system morphology. AM fungi are well noted for their effects on root morphogenesis and can alter the number, length and size of roots, although their modifications to lateral roots seem to be the most frequent effect (Fusconi 2014). Lateral roots in host plants (such as *Medicago*) are induced by recognition of AM fungi lipochitooligosaccharides (LCO) compounds, although both LCO and chitooligosaccharide (CO) compounds can induce them in rice (see Sect. 4.1.4, Maillet et al. 2011; Sun et al. 2015). *Trichoderma* spp. also induces the production of lateral roots and other endophytic fungi cause changes in root diameter and root hair length (Malinowski and Belesky 1999; Contreras-Cornejo et al. 2009). Ectomycorrhizal (EcM) fungi, such as *Laccaria bicolor* (Table 2) that grow intercellularly rather than intracellularly, stimulate lateral root formation and increase root hair length through release of volatile organic compounds and modulation of auxin gradients during the pre-infection stage (Sect. 4.1, Felten et al. 2009; Ditengou et al. 2015). Detrimental microbes can induce similar effects to beneficial microbes on roots, as *A. euteiches* induces lateral root formation in *M. truncatula* during infection (Djebali et al. 2009). *Pythium ultimum* and *Pythium irregulare* infections, however, lead to a smaller root system size and reduced degree of root branching (Larkin et al. 1995).

3.3 Secondary Metabolite Responses

Phytoalexins (PAs) are diverse low molecular weight antimicrobial compounds. Plants produce PAs, most notably after pathogen attack, although beneficial microbes also stimulate their production and this can provide resistance to subsequent infections by pathogenic microbes. Most evidence of these effects is derived from studies on root colonisation effects on aboveground rather than belowground tissues. For example, AM fungi, especially *Funneliformis mosseae*, stimulate capsidiol PA production in pepper stems (Ozgonen and Erkilic 2007). Supporting a role for AM fungi-based protection of belowground tissues, *F. mosseae* colonisation also provides a bioprotector effect against *Phytophthora parasitica* infection in tomato roots (Pozo et al. 2002). Endophytes also induce PA production. A type II hydrophobin protein produced by *Trichoderma longibrachiatum* induces the production of the PA rishitin in tomato leaves (Ruocco et al. 2015). Interestingly the induction of secondary metabolite compounds may be host and/or microbe specific as a different species of *Trichoderma* was shown to suppress expression of genes involved in the production of the PA vestitol in *Lotus japonicus* (Masunaka et al. 2011).

Microbes have evolved to utilise the production of secondary metabolites to their benefit. For example, *Phytophthora sojae* is attracted to soybean roots that exude

isoflavone compounds and *Aphanomyces cochlioides* zoospores display a homing response to host-specific signals (Morris and Ward 1992; Islam and Tahara 2001). Chemicals released by plant roots also help orient the spores of fungi and oomycetes so they do not germinate in the wrong direction away from the host (Deacon 1996). Other compounds, such as flavonoids, may regulate initial stages of AM fungal colonisation and influence hyphal growth and branching, while in pathogenic interactions they are implicated in inhibition of growth (see chapter "Mitigating Abiotic Stresses in Crop Plants by Arbuscular Mycorrhizal Fungi", Hassan and Mathesius 2012 and references therein).

3.4 Systemic Responses to Microbial Colonisation

Colonisation of roots by detrimental microbes can inhibit growth and development of shoots. Conversely, colonisation of roots by beneficial microbes can induce systemic responses such as increases in shoot biomass and greater abiotic and biotic stress resistance in aerial plant tissue. This indicates that root responses to local microbial interactions induce signalling to influence the shoot. AM fungi, *Trichoderma* spp., *P. indica* and DSE interactions (which can all aid nutrient uptake) confer increases in shoot biomass in some plant species (Ozgonen and Erkilic 2007; Fakhro et al. 2010; Andrade-Linares et al. 2011b; Maag et al. 2014). While such growth increases are probably due to the improved nutrient situation of the plant, other systemic responses, such as increased stress tolerance, are conferred by microbe-induced increases in antioxidative capacity through regulation of genes involved in oxidative stress (Brotman et al. 2013). Interestingly, the AM fungus *Rhizophagus irregularis* confers a growth reduction in the non-mycorrhizal plant *A. thaliana*, again highlighting that root–microbe interactions are dependent on the specific organisms involved (see as well Sect. 3.5, Veiga et al. 2013).

As could be expected, signalling between above- and belowground plant tissues during microbial interactions also works in the other direction—microbial colonisation of leaves influences plant roots. For example, colonisation of bean roots with AM fungi was reduced if plant leaves were infected with the pathogen *Colletotrichum gloeosporioides* (Ballhorn et al. 2014).

3.5 Host-Dependent Responses

The outcome of root–microbe interactions can depend on the plant host. Whereas the majority of plants that form interactions with AM fungi form a beneficial symbiotic relationship, in the case of non-mycorrhizal species, the fungi may actually exert a detrimental effect. This indicates that the response of roots to a particular microbe and the outcome of an interaction are case-specific depending on the host and microbe involved. For example, the interaction of AM fungi with

A. thaliana results in root colonisation without arbuscule formation and plant growth is reduced (Veiga et al. 2013). Additionally, the interaction with *Trichoderma* spp. can be swung from neutral endophytic to detrimental depending on the host genetic background (Tucci et al. 2011). Encouragingly, these results suggest that the interaction with these microbes, and the benefits they induce, could be improved through breeding. Finally, the colonisation strategy and lifestyle of *P. indica* also varies in a host-dependent manner, specifically depending on the availability of nitrogen in colonised tissue (Lahrmann et al. 2013). The root responses of these specific individual interactions are likely very different and therefore need to be studied on a case-by-case basis.

4 Parallels in Molecular and Cellular Responses to Beneficial and Detrimental Microbes

To assess parallels in root responses to beneficial and detrimental filamentous microbes, it is pertinent to consider the similarities and differences in their infection strategies and colonisation of root tissue. In order to facilitate effective growth in the plant host, different filamentous microorganisms must perceive chemical and physical signals from the host and modify their growth accordingly. There are different microbial colonisation stages at which root responses can be considered. These are pre-infection (Sect. 4.1), the targeting of microbes to roots and microbial recognition by the root; penetration (Sect. 4.2), root responses to microbial attachment and surface invasion of the host; accommodation (Sect. 4.3), the housing of specialised microbial structures in plant cells; and collaboration or eviction (Sect. 3), the overall response to the interaction, which can be for better or for worse for the plant host.

4.1 Pre-infection

Regardless of whether the outcome of the interaction is beneficial or detrimental, both host plant and invading filamentous microbes release signals signifying their presence in the soil. There is substantial overlap in root responses to these signals, which involve activation of plant defences, but beneficial microbes also produce additional signals to induce symbiosis-related responses in the plant.

4.1.1 Transcriptional Responses Preceding Microbial Contact

In *M. truncatula*, expression of the GRAS transcription factor encoding gene, *RAM1*, is induced before physical contact is made with the AM fungus

R. irregularis and *RAM1* is required for mycorrhizal colonisation and arbuscule formation. However, it is not required for colonisation by the pathogenic oomycetes *P. palmivora* or *A. euteiches* (Gobbato et al. 2013). RAM1 regulates the expression of *RAM2*, a gene encoding a glycerol-3-phosphate acyl transferase, involved in cutin biosynthesis. Later in the mycorrhizal interaction, both *RAM1* and *RAM2* expressions are induced (Gobbato et al. 2012). RAM2 function is important for colonisation of *M. truncatula* roots by *R. irregularis*, *P. palmivora* and *A. euteiches* (Wang et al. 2012; Gobbato et al. 2013). The AM fungi *R. irregularis* and the oomycete pathogen *P. palmivora* both recognise cutin monomers from plant roots as a signal to promote formation of their respective penetrations structures (Table 2). Consequently, colonisation of *ram2-1* plants by *R. irregularis*, *P. palmivora* and also by *A. euteiches* was reduced (Wang et al. 2012; Gobbato et al. 2013).

4.1.2 Responses to the Microbe-Associated Molecular Pattern Chitin

Filamentous microbes display their presence to plants by the release of microbe-associated molecular patterns (MAMPs) (Newman et al. 2013 and references therein). Typically, the presence of true fungi is announced when chitin polymers are released from fungal cell walls by the activities of plant chitinases (Kaku et al. 2006; Silipo et al. 2010). While oomycete cell walls are mainly cellulosic, evidence indicates that chitin is also integral to the cell wall structure of at least some groups of root-infecting oomycetes—*A. euteiches*, for example (Badreddine et al. 2008; Nars et al. 2013a). In *M. truncatula*, chitinase expression in roots was induced by interaction with microbes with different lifestyles. Interestingly, the AM fungi tested induced some different chitinases compared to the pathogens, indicating there may be microbe–lifestyle-specific effects for these enzymes (Salzer et al. 2000).

Most work on chitin perception has been conducted in suspension-cultured rice cells (Kaku et al. 2006; Kishimoto et al. 2010; Shimizu et al. 2010; Kouzai et al. 2014). Preferential recognition of octameric chitooligosaccharide polymers (CO8, chitin) at the plant cell surface triggers a cascade of downstream signalling leading to the activation of plant defence responses (Hamel and Beaudoin 2010; Shimizu et al. 2010). The lysin motif (LysM)-containing proteins OsCERK1 and OsCEBiP are required for pathogen chitin recognition in rice, where they function as a heterodimer (Miya et al. 2007; Liu et al. 2012; Shimizu et al. 2010). On binding CO8 from filamentous microbes, OsCEBiP recruits OsCERK1 that then phosphorylates OsRacGEF1, enabling the activation of signalling pathways that lead to activation of MAPK cascades and the production of reactive oxygen species, PAs (Sect. 3.3), lignins and pathogenesis-related proteins in rice (see Sanchez-Vallet et al. 2015). Similarly in *M. truncatula* roots, chitin fractions induced the production of extracellular reactive oxygen species and the transient expression of defence-associated genes (Nars et al. 2013b).

$[Ca^{2+}]_{cyt}$ increases are also observed in response to MAMP recognition. The use of $[Ca^{2+}]_{cyt}$ elevation mutants has demonstrated the importance of this response for

P. indica-mediated growth promotion in *A. thaliana* (Vadassery and Oelmuller 2009; Vadassery et al. 2009). *P. indica* induces different $[Ca^{2+}]_{cyt}$ responses in tobacco, suggesting there are host species-specific responses to the same microbe (Vadassery and Oelmuller 2009). *T. atroviride* and AM fungi culture exudates were also found to increase $[Ca^{2+}]_{cyt}$ levels (Navazio et al. 2007). Therefore, Ca^{2+} responses in roots are a common feature of interactions with both detrimental and beneficial microbes (see also Sect. 4.1.4).

Recently, *OsCERK1* was shown to be required for colonisation by AM fungi in rice roots, as well as for pathogenic *M. oryzae* colonisation in leaves (Zhang et al. 2015). OsCEBiP, the interacting partner of OsCERK1 in chitin perception, does not appear to play a role in mycorrhization, as the colonisation phenotype of mutant *cebip* plants was normal (Miyata et al. 2014). However, OsCEBiP is important for resistance to the fungal pathogen *M. oryzae* in leaves (Kishimoto et al. 2010; Mentlak et al. 2012; Kouzai et al. 2014). This implies, therefore, that there are different *OsCERK1*-dependent signalling complexes responsible for the detection of different microbes (Table 1). Both *OsCERK1* and *OsCEBiP* are expressed in rice roots; however, crucial information is still missing about the role of these genes in pathogen infection in this plant tissue (Shimizu et al. 2010).

4.1.3 Oomycete Elicitins

Phytophthora and *Pythium* oomycete pathogens also produce elicitin MAMPs (structurally conserved extracellular proteins with lipid binding roles) that trigger plant immunity. Plant recognition of elicitin proteins has only recently been described. The elicitin response (ELR) receptor-like protein was identified in a wild potato species and mediates extracellular recognition of a conserved pathogen elicitin domain in leaves (Du et al. 2015). Again it remains to be shown whether ELR is important for defence responses upon recognition of elicitins in roots.

4.1.4 Responses to Short (Lipo)chitooligosaccharides

In addition to the release of MAMPs, AM fungi also produce MYC factors which are diffusible lipochitooligosaccharide (LCO) and short-chain chitooligosaccharide (CO) signals that promote symbiosis-related responses in host–plant roots (Maillet et al. 2011; Genre et al. 2013). LCOs are mostly tetrameric or pentameric, β-1-4 linked *N*-acetylglucosamine chitooligosaccharide backbones decorated with various chemical groups, including sulphates, while short-chain COs are undecorated (Gough and Cullimore 2011; Genre et al. 2013; Maillet et al. 2011; Oldroyd 2013). AM fungal LCOs promote lateral root development (see Sect. 3.2) and enhance the formation of mycorrhizal symbiosis in *Medicago* but stimulate symbiosis-related nuclear Ca^{2+} spiking (an early event in the development of symbiosis) less efficiently than short-chain COs (Genre et al. 2013).