

PREFACE

Mycorrhizae represent a symbiotic relationship between a fungus and plant roots. The fungus colonizes the roots of the host plant, either intracellularly in arbuscular mycorrhizal fungi, or extracellularly in ectomycorrhizal fungi. Host plants enhance the defense of mycorrhizal fungi against fungivores. Mycorrhizae mediate host plant-insect interactions. Arbuscular mycorrhiza play a crucial role in augmenting plant resistance to stressful conditions like diseases, drought, heavy metal toxicity, high temperatures, salinity, and waterlogging. They are also important in enhancing decomposition of toxic organic substances, in remediating polluted environments, and in keeping a stable ecosystem and sustainable productivity. This book discusses the structure, development and function of mycorrhizas.

Chapter 1 – The establishment and the maintenance of a mutualistic arbuscular mycorrhizal (AM) interaction are based on intense communications between the partners and within and between plant cells and organs. Legumes, which can in addition to the AM symbiosis undergo a symbiosis with nitrogen-fixing rhizobacteria, serve as model plants to study the signaling in both root endosymbioses. This chapter will summarize the current knowledge on early signaling and regulation of arbuscular mycorrhization as follows. The first part provides an overview on the signaling leading to successful infection of the root – including common elements required for both legume endosymbioses. This part comprises (i) signals involved in attraction and recognition of the partners, (ii) the activation of a signaling cascade in root hair cells of legumes by AM fungal and rhizobacterial signals, and (iii) the mechanisms leading to successful penetration of the root surface and to intracellular accommodation of the AM fungal partner. The second part focuses on the carbon supply of the fungus and on the role of apoplastic invertases and sugar-induced signaling in

the AM interaction. The regulation of the AM symbiosis by a long-distance and systemic feedback mechanism, called autoregulation of mycorrhization, is topic of the last part of this chapter.

Chapter 2 – Most plant species associate with mycorrhizas to perform better under stressful conditions and the increasing recognition of the mycorrhizal interactions, especially arbuscular mycorrhizal fungi (AMF) may have important functions in terrestrial ecosystems. AMF structures such as total root colonized and percent root length colonized by arbuscules can be used to estimate of AMF abundance within a plant or ecosystem. Also important is to evaluate the extraradical hyphae of AMF that extend into the surrounding soil much further than do roots. Due to their strategic position at the soil–root interface, the study of AMF in different undisturbed or degraded ecosystems can contribute to better understand the effect of global change on plant-soil associated communities. Many plant species in tropical regions where semiarid environments are also found associate with AMF and this symbioses has to be monitored under global change. This chapter explores the current available information relevant to benefits from AMF symbioses in natural and disturbed sites. Possible functions of AMF structures and research on Glomalin and AMF interaction with biochar are also discussed. Better knowledge of the wide variation in AMF parameters is important for understanding the ecology of different land ecosystems and for management purposes.

Chapter 3 – Mycorrhizae represent a symbiotic relationship between a fungus and plant roots. The fungus colonizes the roots of the host plant, either intracellularly in arbuscular mycorrhizal fungi, or extracellularly in ectomycorrhizal fungi. Host plants enhance the defense of mycorrhizal fungi against fungivores. Mycorrhizae mediate host plant-insect interactions.

Arbuscular mycorrhizal exudates are important to the formation of lateral roots, and in the absorption and accumulation of elements including manganese, phosphorus, potassium, and zinc. Mycorrhizae alter nitrogen acquisition and improve plant growth. Organic nitrogen is more accessible to ectomycorrhizal and ericoid mycorrhizal fungi than arbuscular mycorrhizal fungi because of the presence of enzymes capable of degrading nitrogen-containing molecules in the former type of fungi. Ectomycorrhizal fungi are involved in decomposing and mobilizing nitrogen from organic matter-protein complexes in forest soils. Ectomycorrhizae induce changes in the surface structure and composition of soil colloids.

Mycorrhizae are involved in phytoremediation of soil contaminated with heavy metals. They also enhance plant tolerance to adverse conditions,

decrease aluminium phytotoxicity in acidic soils, and improve plant antioxidant systems and tolerance to salinity.

Chapter 4 – In soil N is present in inorganic forms as ammonium (NH_4^+) and nitrate (NO_3^-), and as organic compounds such as amino acids, peptides and proteins. However, availability of N appears to be a limiting factor for forest tree growth in most temperate and boreal ecosystems where a significant pool of N is present in complex organic compounds.

Ectomycorrhizal fungi play a major role in soil N cycling by their utilization of both inorganic and organic forms. Nutrients, including N, are mobilized from soil by fungal mycelia and transferred to the host plant.

On the other hand, the host feeds the fungal symbiont with carbon compounds derived from photosynthesis. Despite the widely accepted importance of ectomycorrhizas on forest tree nutrition and N cycling, the control of basic N metabolism in ectomycorrhizal fungi is still poorly studied. Therefore, resolving how ectomycorrhizal fungi regulate their N acquisition will contribute to a better understanding of forest tree nutrition. *Laccaria bicolor* is an ectomycorrhizal basidiomycete which forms symbiosis with several boreal and temperate forest trees such as birch, pine and poplar.

Ectomycorrhizal fungi such as *L. bicolor* compete with saprotrophs and plants for the limited pool of nutrients. Ectomycorrhizal fungi are well known to utilize complex organic N sources especially abundant in acid boreal and temperate forest soils but mineral N is efficiently utilized as well. Ectomycorrhizal fungi appear to be well adapted to low mineral N concentrations and efficiently assimilate inorganic N as NH_4^+ , NO_3^- , the first generally being the preferred N source. While NH_4^+ is the dominant mineral N form in undisturbed boreal and temperate forest soils the true availability of NO_3^- for ectomycorrhizal fungi in their habitat is not well established. The majority of studied ectomycorrhizal fungi shows a conserved capacity to grow on NO_3^- as the sole N source and some of them have even been reported to prefer NO_3^- over NH_4^+ . This suggests that NO_3^- may play a more significant role as N source in forest ecosystems as traditionally believed. *Laccaria*, like many other ectomycorrhizal fungi, clearly prefers NH_4^+ but it also grows well on NO_3^- . Its genome encodes for one copy of each NO_3^- assimilation genes: the transporter *Lbnrt*, the NO_3^- -reductase *Lbnr* and the NO_2^- -reductase *Lbnir*. The genes are organized in the so-called fungal high-affinity N-assimilation cluster (fHANT-AC). The authors found massive effects on the formation of ECM root tips of poplar when N-assimilation in *L. bicolor* was impaired by silencing of a selected NO_3^- -metabolism gene, the nitrate reductase encoding gene *Lbnr*. Alteration of the symbiotic capacity was specific to the RNAi-

induced reduction in N assimilation of the fungal partner. Silencing of *Lbnr* strongly impaired mycorrhiza formation on NO_3^- but not on NH_4^+ . The participation of NO_3^- -utilization genes in the symbiotic interaction of *Laccaria* is hence clear.

The authors have extended the nitrate utilization RNAi-based gene silencing studies of the ECM basidiomycete *Laccaria bicolor* by altering the expression of *LbNrt*, the sole nitrate transporter encoding gene of the fungus. Here they report the first nutrient transporter mutants for mycorrhizal fungi. Silencing of *LbNrt* results in fungal strains with minimal detectable *LbNrt* transcript levels, significantly reduced growth capacity on nitrate and altered symbiotic interaction with poplar. Transporter silencing also produces co-downregulation of the whole fHANT-AC. Moreover, this effect on the nitrate utilization pathway seems to be independent of extracellular nitrate or the nitrogen status of the fungus. The authors' results show a novel and key nitrate uptake-independent regulatory role for a eukaryotic nitrate transporter. The possible cellular mechanisms underneath this regulation are discussed in the light of the current knowledge on NRT2-type nitrate transporters.

Chapter 5 – The capacity of roots to sense soil physicochemical parameters plays an essential role in maintaining plant nutritional and developmental functions under abiotic stress. Drought greatly affects the growth and development of plants and can cause extensive losses to agricultural productivity. The water regime affects a wide variety of physiological and biochemical processes in plants including an increased production of reactive oxygen species (ROS) capable of causing oxidative damage to proteins, DNA and lipids. Moreover, this ROS production can induce cellular, anatomical, and morphological changes that improve drought tolerance. Arbuscular mycorrhizal fungi (AMF) colonize a wide range of plant species though the ability of different AMF to promote host growth and/or water deficit resistance is variable. AMF contributes to drought tolerance through a combination of physical, nutritional and physiological effects. The response to oxidative stress depends on many factors, including the organism tissue and the degree of stress. In most cases mycorrhizal plants increase antioxidant defenses, such as the ROS-scavenging enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). AMF can maintain the levels of malondialdehyde (MDA), a compound indicative of lipid damage, by rapidly increasing antioxidant defenses and preventing lipid damage. In other cases an increase of MDA is observed in mycorrhizal plants due to an active response to oxidative stress. A critical step of AMF inoculation technology is the appropriate selection of fungal isolates to be used

as inoculants. Nursery cultivation is the most convenient way to improve the success of semi-woody cuttings transplantation (e.g. olive, pomegranate, citrus) at the crop area. Plants suffer at least two transplant moments in nursery conditions prior to outside cultivation. AMF can reduce transplant stress by changing the morphology of the root system favouring the establishment of plants. Thus AMF can greatly contribute to crop productivity and environmental sustainability. Artificial inoculation of plant cuttings with AMF at an early stage of cutting development has been adopted by an increasing number of nursery managers as a method for promoting growth, production and precocity. Moreover, it is an essential component for most plants and it can be used as a biofertilizer resource. In this chapter the authors discuss the effect of an early AMF inoculation in alleviating oxidative stress in semi-woody cutting propagation management under nursery conditions. They also discuss the benefits of AMF to solve the problematic issue of water deficit and transplant stresses generated during plant growth.

Chapter 6 – This article reviews the state of the art with respect to the environmental effects of wastewater irrigated agriculture on arbuscular mycorrhizal fungi in arid and semi-arid regions on a field scale. Petroleum, tannery and pharmaceutical compounds emerge as pollutants when wastewater is used for irrigation without any previous treatment, situation which is now the object of new studies. In the last few decades, the amounts of detergents, fats, oils, heavy metals and synthesized organic compounds generated by industrial activities have increased, and some 10,000 new organic compounds are added each year, and released into the environment for direct or indirect application over a long period of time. In both developed and developing countries, the most prevalent practice is the application of municipal wastewater (both treated and untreated) to land. Impact from wastewater on agricultural soil, is mainly due to the presence of high nutrient contents (Nitrogen and Phosphorus), high total dissolved solids and compounds pollutants, which are added to the soil over time. Arbuscular mycorrhizal plants have two pathways that can contribute to nutrient uptake from soil; direct uptake from the rhizosphere by root epidermal cells and the vesicular arbuscular mycorrhizae uptake pathway via the fungi. The establishment of the vesicular arbuscular mycorrhizae symbiosis can be disturbed by environmental conditions, including phosphorus (P) availability which can inhibit the symbiotic interaction. Interestingly, recent studies have demonstrated a cross-talk between phosphorus and nitrogen availabilities to control arbuscular mycorrhizal associations, thus indicating a high level of integration of mycorrhizal responses to mineral nutrition. Some authors have

reported a direct effect of P on arbuscular mycorrhizal spore germination and hyphal growth. Evidence has also been gathered for downregulation by P of well established vesicular arbuscular mycorrhizae interactions, via a reduced production of root compounds. Arbuscular mycorrhizal symbiosis could be arrested almost completely by a high P supply at a very early stage, prior to the attachment of the fungus on the root. The synthesis and exudation of strigolactones are negatively affected by a high availability of P. Under conditions of P sufficiency, a strong reduction of strigolactone release would prevent the stimulation of AM fungi and hence the establishment of the interaction. The impact of arbuscular mycorrhizas, is starting to draw attention in the context of wastewater irrigation and phytoremediation of organics, and recent results have shown that these symbioses may be crucial for both plant establishment on contaminated sites.

Chapter 7 – *Fusarium* root rot tolerance and changes in antioxidative ability in arbuscular mycorrhizal fungi (AMF) [*Glomus intraradices* (Gi) and *Gigaspora margarita* (GM)] and non-pathogenic *Fusarium* (NPF) [*Fusarium oxysporum* f. sp. *asparagi* (Foa)]-inoculated asparagus plants (*Asparagus officinalis* L., cv. 'Welcome') were investigated. Dry weight of shoots and roots increased in AMF and NPF-inoculated plants. Though, dry weight increased in NPF-inoculated plants compared than control, which is comparatively lower than AMF-inoculated plants. On the other hand, SOD activity, APX, DPPH radical scavenging activity, content of polyphenol and ascorbic acid increased under inoculation of pathogen stress condition; these effects differed between shoots and roots. As for disease tolerance, the incidence and severity of symptom was less in both AMF and NPF-inoculated asparagus plants than non-inoculated plants, though the effect differed between Gi and GM inoculated asparagus plants with or without presence of NPF. These results suggest that tolerance to *Fusarium* root rot was increased by AMF colonization with or without NPF in asparagus plants. As well as, antioxidative ability increased in pathogen-stressed conditions, so that the *Fusarium* root rot tolerance might be associated with the increase in such antioxidative factors.