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The role of ectomycorrhizas in heavy metal stress tolerance of host plants

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ABSTRACT

Ectomycorrhizas (EMs) are mutualistic associations between certain soil fungi and higher plants. EMs can modulate the cellular, physiological and molecular processes of host plants, resulting in altered responses of the colonized plants to heavy metals. Progress in elucidating the role of EMs in modulating heavy metal tolerance of host trees is reviewed. In the last decade, a number of ectomycorrhizal fungal isolates and host plants have been characterized for their tolerance to heavy metals. Additionally, the cellular processes have been investigated with regard to heavy metal uptake, transport, distribution, toxicity and detoxification by ectomycorrhizal fungi and/or host plants. At the cellular level, mechanisms of heavy metal detoxification include (i) binding of heavy metals to cell wall and extracellular exudates, (ii) decreased uptake and/or pumping metal ions out of cytosol, (iii) chelation of metal ions in cytosol, (iv) compartmentation of metals in vacuoles or other subcellular structures, and (v) repair of damaged biomolecules. The efficiency of these protective measures is often increased by EMs, resulting in improved physiological status and rescued growth. While physiological and cellular responses to heavy metals have been well studied, experimental data on the underlying molecular mechanisms, especially those induced by the interaction of ectomycorrhizal fungi and hosts, are scattered. Progress in genome sequencing of EM partners has revealed the importance of metal transporters in mediating tolerance. A better understanding of the molecular mechanisms is essential for effective application of selected fungal isolates and hosts to improve the efficiency of bioremediation on heavy metal polluted sites.

1. Introduction

1.1. Function and ecological significance of ectomycorrhizas

The ectomycorrhiza (EM) is an association formed between ectomycorrhizal fungi (EMF) and the root tips of many plant species (Smith and Read, 2008). Most EMF belong to the basidiomycota or ascomycota and it has been estimated that as many as 10 000 fungal species can form EMs with host plants (Finlay, 2008). The hosts of EMF are mainly tree species in the families *Pinaceae*, *Fagaceae*, *Dipterocarpaceae* and *Caesalpinoidaceae*, distributed in tropic, subtropical, temperate and boreal forests (Smith and Read, 2008).

In functional EMs, fungal hyphae ensheath the root tips to form the hyphal mantle; inside the mantle at the root surface hyphae penetrate into the cell wall and grow between epidermal cells and cortical cells, but never enter the cell lumen of roots (Bonfante and Genre, 2010). These hyphae form the Hartig net, the interface for exchange of water, nutrients, and other compounds between both partners (Nehls et al., 2010). Outside the fungal mantle, hyphae extend into the forest soil and have access to nutrients unavailable to plant roots. Thus, the ectomycorrhizal plants obtain water and nutrients via the fungal hyphae from the soil, while the fungi are rewarded with photosynthates or stored carbohydrates of the host plants (Druebert et al., 2009; Kaiser et al., 2010; Pena et al., 2010). In forests and plantations, the roots of a distinct tree species are colonized by many different EMF (Buee et al., 2005; Danielsen et al., 2012, 2013; Lang et al., 2010, 2013). Different trees species can be interconnected by the fungal hyphae which form a common ectomycorrhizal network (Bingham and Simard, 2012b; Martin et al., 2007; Martin and Nehls, 2009; Rooney et al., 2009). With the help of this common ectomycorrhizal network, exchange of water and nutrients between different individual tree can occur, which may increase the stability and fitness of forest ecosystems against adverse environments (Bingham and Simard, 2011, 2012a).

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Trees are long-lived perennial plants and, therefore are likely to face abiotic and biotic stresses including nutrient deficiency, drought, salinity, heavy metals and pests during their life time (Campioli et al., 2012; Chen and Polle, 2010; Osakabe et al., 2011). For instance, EMs mediate improved drought tolerance of their host plants (Beniwal et al., 2010, 2011; Luo et al., 2009b; Pena et al., 2013; Pena and Polle, 2013). EMs can furthermore attenuate salt stress on host plants (Langenfeld-Heyser et al., 2007; Li et al., 2012; Luo et al., 2009a, 2011). Positive effects of EMs on host resistance to pests and/or pathogens have been unraveled (Gange et al., 2005; Hartley and Gange, 2009; Nerg et al., 2008, 2009; Pritsch et al., 2009). EMs have positive effects on plant survival, even in the absence of a functional symbiosis (Müller et al., 2013). Recent reviews have focused on mycorrhizal modulation of host drought and pathogen responses (Hartley and Gange, 2009; Lehto and Zwiazek, 2011; Muller et al., 2007b). Progress has also been reviewed in the field of mycorrhiza-modulated host plant tolerance to heavy metals (Colpaert et al., 2011; Rajkumar et al., 2012). However, there is rapid progress in the area of the cellular, physiological and molecular mechanisms underlying the function of EMs and ectomycorrhizal influence on plant heavy metal tolerance. Therefore, we will focus this review mainly on these aspects of the biotic (EMF) and abiotic (heavy metals) interaction of plants.

1.2. Sources and toxicity of heavy metals

Heavy metals are elements with a density higher than 5 g cm^{-3}, mainly including transition metals, some metalloids, lanthanides, and actinides (Schützendübel and Polle, 2002). Not all of these heavy metals are biologically important. Among these metals, some elements such as Zn, Cu and Mn, are required as micronutrients, but higher concentrations of these heavy metals can be toxic (Ducic et al., 2006; Ducic and Polle, 2007; Langer et al., 2009). Other heavy metals such as Cd, As (a metalloid, but hereon also included as a heavy metal), Pb, Hg and Ni, are non-nutritional and toxic elements for most organisms (He et al., 2011, 2013a; Jourand et al., 2010; Krupa and Kozdroj, 2007).

Heavy metals occur naturally in soil with large variations in their concentrations. Notably, they are steadily accumulating in the environment because of the rapid expansion of industrial activities and their non-degradability (Clemens et al., 2013). Because of their toxicity, the enrichment of heavy metals, particularly of the ions of heavy metals, leads to decreases in biodiversity and productivity and, thereby, results in changes in structure and function of ecosystems (Mayor et al., 2013; Niemeyer et al., 2012; Pauwels et al., 2008). Heavy metals in soils can be taken up by plants and accumulate in edible parts, which may eventually enter the human body through the food chain (Kaplan et al., 2011). Some heavy metals are carcinogenic and toxic, affecting the central nervous system (i.e., Mn, Hg, Pb, As), the kidneys or liver (i.e., Hg, Pb, Cd, Cu), skin, bones and teeth (i.e., Ni, Cd, Cu) (Bertin and Averbeck, 2006). Thus, toxic heavy metals in ecosystems pose a serious threat to human health.

The typical concentrations ($\mu g\,g^{-1}$) of heavy metals in most plants are 50 for Zn, 10 for Cu, 200 for Mn, 0.05 for Cd, 0.1 for As, 1 for Pb and 1.5 for Ni (van der Ent et al., 2013). In contrast, plants with foliar concentrations ($\mu g\,g^{-1}$) of heavy metals above 10,000 for Zn and Mn, 1000 for Cu, As, Pb and Ni and 100 for Cd are regarded as hyperaccumulators for the respective element (van der Ent et al., 2013). Thereby, plants in concert with EMF and other microbes can be used for bioremediation of heavy metal polluted soils (Rajkumar et al., 2012). For bioremediation, a particular challenge is to obtain hyperaccumulating plants and/or micro-organisms including EMF which can readily absorb high amounts of heavy metals and transport them to the harvestable parts. In the past, numerous herbaceous hyperaccumulators of heavy metals have been identified, such as *Phytolacca acinosa* and *Maytenus founieri* for Mn (Fernando et al., 2008; Xue et al., 2004), *Solanum nigrum* and *Sedum alfredii* for Zn (Ferraz et al., 2012; Lu et al., 2013), *Commoelina communis* and *Elsholtzia splendens* for Cu (Jiang et al., 2004; Wang et al., 2004), *Arabidopsis halleri* and *Noccaea caerulescens* (formerly known as *Thlaspi caerulescens*) for Cd (Hanikenne et al., 2008; Kramer, 2010; Milner and Kochian, 2008), *Pityrogramma calomelanos* and *Pteris vittata* for As (Huang et al., 2011; Visoottiviseth et al., 2002), *Microstegium ciliatum*, *Polygala umbonata*, *Spermacoce mauritiana* and *Hirschfeldia incana* for Pb (Auguy et al., 2013; Rotkittikhun et al., 2006), *Sesbania drummondii* and *Brassica juncea* for Hg (Shiyab et al., 2009; Venkatachalam et al., 2009), and *Alyssum inflatum*, *Rinorea bengalensis* and *Pimelea leptospermoides* for Ni (Ghasemi et al., 2009; van der Ent et al., 2013).

However, herbaceous plants produce limited amounts of aboveground biomass which constrains the amount of absorbed metals. Furthermore, their root system is also less developed than that of woody species, which limits the access to heavy metals in deeper soil layers. Therefore, application of fast growing woody plants, such as *Populus* and *Salix* species, which have large biomass, deep root systems and can sequester intermediate heavy metal concentrations in aerial parts, has been proposed for phytoremediation (He et al., 2013b; Merkle, 2006; Vollenweider et al., 2006; Zhao and McGrath, 2009). Roots of poplars and willows form EMs with ectomycorrhizal fungi (Baum and Hrynkiewicz, 2005; Baum et al., 2006, 2009; Danielsen et al., 2012, 2013; Krpata et al., 2008, 2009; Mrnka et al., 2012; Sell et al., 2005) and therefore not only the performance of trees on polluted soils but also their associated microbes are of high importance for the success of bioremediation. In combination with appropriate ectomycorrhizal fungi, trees can play a key role in bioremediation of heavy metal polluted soils (Aggangan and Aggangan, 2012; Krpata et al., 2009; Sousa et al., 2012). Readers interested in general aspects of bioremediation of plant-associated microbes, are referred to several recent excellent reviews (Clemens et al., 2013; Kramer, 2010; Milner and Kochian, 2008; Rajkumar et al., 2012). Here, we wish to highlight that the interaction of EMF with trees that affects plant tolerance to heavy metals also has practical applications and that an improved understanding of the underlying mechanisms of this abiotic × biotic interaction contributes to developing improved soil amelioration strategies. Furthermore, we specifically distinguish the effects of nutritional (Zn, Cu and Mn) and non-nutritional toxic (Cd, Pb, Hg, Ni and As) heavy metals.

2. Effects of EMs on nutritional heavy metals in plants

2.1. Uptake, transport and distribution of nutritional heavy metals

As micronutrients Zn, Cu and Mn are needed in minute amounts by ectomycorrhizal fungi and plants. The nutrients usually occur in ionic forms in soil solution or are adsorbed to soil particles and are taken up by specific transporters localized in the plasma membrane and the internal membrane system of fungal and root cells (Martin et al., 2008; Pilon et al., 2009). Genome sequencing of *Laccaria bicolor* resulted in the discovery of a number of genes encoding putative transporters for Zn, Cu and Mn ions (Martin et al., 2008). To date, only few nutritional heavy metal transporters have been functionally studied in EMF. For instance, in the ectomycorrhizal fungus *Hebeloma cylindrosporum*, a gene (*HcZnT1*) conferring Zn tolerance was isolated and functionally characterized (Blaudez and Chalot, 2011). HcZnT1 was constitutively expressed in cells of *H. cylindrosporum* irrespective of the external Zn status and the cell type (mycelia, sporocarps or ectomycorrhizas) (Blaudez and Chalot, 2011). Further characterization showed that HcZnT1 was probably located in the endoplasmic reticulum (ER) membranes (Blaudez