# O-coumaric acid ester, a potential signaling molecule detected during early *in vitro* co-culture between *Pinus pinea* plantlets and the ectomycorrhizal fungus *Pisolithus arhizus*

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## Abstract

In ectomycorrhizal symbiosis, plant roots and fungi function together as a unit. Previously, we showed improvement in various root parameters during *in vitro* co-culture of *Pinus pinea* and *Pisolithus arhizus* before physical contact occurred, as well as increased survival rate of the inoculated plantlets during acclimatization. To better understand the positive effects on root growth parameters before the establishment of physical contact between these partners, the biochemical compounds released to the liquid phase of the co-culture medium were analysed. It is known that biochemical signals lead to the development of complex structures in both the plant and the fungus that constitute an ectomycorrhiza. The results of HPLC-UV and LC-DAD-MS analysis of the liquid phase medium samples that were collected from 1 to 10 days of *in vitro* co-culture are presented. O-coumaric acid ester, a phenolic compound, was identified in root exudates of stone pine from the second day and its presence was detected for up to 10 days of co-culture. This result contributes to the understanding of the role of phenolic compounds in pine/ ectomycorrhiza symbiosis establishment and also explains some of our previous results.

**Keywords** Adventitious roots, ectomycorrhiza, phenolics, stone pine, symbiosis

## INTRODUCTION

Ectomycorrhizae (ECM) are symbiotic structures between plant roots and fungi. In the ectomycorrhizal symbiosis, the host (plant roots) and the mycobiont (ectomycorrhizal fungus) function collectively as an entity. The development of ECM in plants frequently allows them to get established in habitats that neither symbiont could occupy independently (Nehls et al. 2000).

ECM development involves a series of complex processes that occur simultaneously in symbionts. Extramatrical hyphae, the mantle and the intraradicular hyphal network are active metabolic bodies that provide essential nutrients (e.g. nitrogen, phosphate) to the host plant and carbohydrates for the fungal partner making this a mutualistic association (Allen 1991; Varma and Hock 1994; Smith and Read 1997; Martin et al. 2001).

Successful colonization of roots by mycorrhizal fungi and further development of the ectomycorrhizal structure, results from a coordinated series of events mediated by biochemical signals (Seddas et al. 2009). During ECM establishment the molecular dialog initiate developments that lead to physical steps in the association once the detection or attraction of the partner occur before physical contact (Harrison 2005).The fungus must face the host defense mechanisms and be able to initiate the mutual nutrient transfer across the root-fungus interface (Reis et al. 2011). This is achieved by an intense cell activity before and after physical contact between partners. In a recent review by Bonfante and Genre (2010) the identification of several novel nutrient transporters has revealed some cellular processes that underlie symbiosis, but the biochemical signals prior to physical contact and their functions still need to be elucidated, especially for ectomycorrhizal fungi.

Martin et al. (2001) suggested that rhizospheric signals including auxins, flavonoids, alkaloids, cytokinins, and other metabolites produced by both partners could act in a synergistic or in antagonistic way. More recently plant phenolic compounds such as p-coumaric acid, coumarin, naringenin and other flavonoids were also cited as potential candidates of signals during mycorrhizal formation (Lynn and Chang 1990; Mandal et al. 2010; Amalesh et al. 2011; Plett and Martin 2012; Hassan and Mathesius 2012). Phenolic compounds are ubiquitous in plants and participate in several important functions which enable them to adapt to changing biotic and abiotic environments (Boudet 2007).

Several studies have shown the benefits of using ECM fungi (*Amanita*, *Hebeloma*, *Laccaria*, *Lactarius*, *Pisolithus*, *Rhizopogon*, *Scleroderma*, and *Suillus*) in conifer micropropagation (Grange et al. 1997; Wallander 2000; Rai 2001; Wu et al. 2003; Taylor et al. 2004; Niemi et al. 2004; Adriaensen et al. 2006). Among many advantages the mycorrhized plants (either with arbuscular mycorrhizal (AM) or ECM fungi) were more efficient in water and nutrient

absorption through an increased area of soil colonization, had increased pathogen resistance and increased transplantation survival compared with non mycorrhized plants. In addition some ECM and ericoid fungi could breakdown phenolic compounds present in the soil that might interfere with nutrient uptake (Allen et al. 1989; Brundrett 1991; Grandmaison et al. 1993; Newsham et al. 1995; Little and Maun 1996; Bending and Read 1997; Cordier et al. 1998; Bratek et al. 2002).

Recently, we demonstrated that *in vitro* co-culture of *Pinus pinea* plantlets with *Pisolithus arhizus* helped to overcome the cessation of adventitious root growth and resulted in a root system that was better adapted to post transplantation stress. None of the inoculated plantlets died in spite of using exclusively sterile vermiculite in the early phase of acclimatization during which a vast mycorrhizal symbiosis was established. Moreover, fewer roots were lost during transplantation which was facilitated by the morphological modifications of the mycorrhized roots such as the presence of the hyphae around the roots and the internal Hartig net, which increased root thickness and contributed to a more robust root system (Ragonezi et al. 2012).

In this study the objective was to characterize the chemical nature of the mediators and the period of *in vitro* co-culture during which the signaling between *P. arhizus* and roots of *P. pinea* occurred. We present biochemical results of high-performance liquid chromatography (HPLC-UV) and a liquid chromatography - diode array detector - mass spectrometry (LC-DAD-MS) analysis of the metabolites released into the liquid phase of the double layer medium during the first days of plant/fungus co-culture.