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Improved zinc tolerance in *Eucalyptus globulus* inoculated with *Glomus deserticola* and *Trametes versicolor* or *Coriolopsis rigida*

C. Arriagada [a,*], G. Pereira [c], I. García-Romera [b], J.A. Ocampo [b]

[a] Dept. Ciencias Forestales, Universidad de La Frontera, Casilla 54-D, Temuco, Chile
[b] Estación Experimental del Zaidín, Profesor Albareda 1, 18008 Granada, Spain
[c] Departamento Forestal, Universidad de Concepción, Los Angeles, Chile

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ABSTRACT

The potential of interactions between saprophytic and arbuscular mycorrhizal (AM) fungi to improve *Eucalyptus globulus* grown in soil contaminated with Zn were investigated. The presence of 100 mg kg^{-1} Zn decreased the shoot and root dry weight of *E. globulus* colonized with *Glomus deserticola* less than in plants not colonized with AM. Zn also decreased the extent of root length colonization by AM and the AM fungus metabolic activity, measured as succinate dehydrogenase (SDH) activity of the fungal mycelium inside the *E. globulus* root. The saprophytic fungi *Trametes versicolor* and *Coriolopsis rigida* increased the shoot dry weight and the tolerance of *E. globulus* to Zn when these plants were AM-colonized. Both saprophytic fungi increased the percentage of AM root length colonization and elevated *G. deserticola* SDH activity in the presence of all Zn concentrations applied to the soil. In the presence of 500 and 1000 mg kg^{-1} Zn, there were higher metal concentrations in roots and shoots of AM than in non-AM plants; furthermore, both saprophytic fungi increased Zn uptake by *E. globulus* colonized by *G. deserticola*. The higher root to shoot metal ratio observed in mycorrhizal *E. globulus* plants indicates that *G. deserticola* enhanced Zn uptake and accumulation in the root system, playing a filtering/sequestering role in the presence of Zn. However, saprophytic fungi did not increase the root to shoot Zn ratio in mycorrhizal *E. globulus* plants. The effect of the saprophytic fungi on the tolerance and the accumulation of Zn in *E. globulus* was mediated by its effect on the colonization and metabolic activity of the AM fungi.

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1. Introduction

Zinc is a micronutrient essential for plant development and growth and is involved in a number of enzymatic reactions catalyzing nucleic acid metabolism. However, excess of Zn can inhibit many plant metabolic functions, resulting in retarded growth and senescence (Marschner, 1995). It is recognized that soil microorganisms such as some saprophytic and arbuscular mycorrhizal (AM) fungi play an important role in plant health, nutrient uptake and tolerance against heavy metals (Heggo et al., 1990; Haselwandter and Berreck, 1994; Arriagada et al., 2005).

The arbuscular mycorrhizal (AM) fungi are a primary component of the microbial biomass in soil. This symbiosis can benefit plant growth, particularly through enhanced phosphorus, water and mineral uptake (Smith and Read, 2008). Mycorrhizal fungi enhance nutrient availability and plant tolerance to the presence of high quantities of heavy metals such as Zn in soil (Gaur and Adholeya, 2004). However, the effect of AM fungi on the uptake of metals by plants is not yet clear. It is rather well established that AM accumulate Zn; hence, they increase Zn absorption and accumulation in the roots (Chen et al., 2003).

Saprophytic fungi are important and abundant components of rhizosphere soil, where they obtain great nutritional benefit from organic and inorganic compounds released from living roots and sloughed cells (Dix and Webster, 1995). Some experimental results confirm the existence of synergistic effects of some saprophytic fungi on plant growth and root colonization by AM fungi in soil contaminated with heavy metals (Arriagada et al., 2004, 2005, 2007). Saprophytic fungi of *Trametes* and *Coriolopsis* genera were found to be tolerant to Zn; they can concentrate this heavy metal in their mycelia (Barajas-Aceves et al., 2002). Although these white-rot fungi effectively degrade various xenobiotics, relatively few studies have been done using these fungi for soil remediation (Baldrian, 2003; Bayramoglu et al., 2003).

Eucalyptus is a tree species exhibiting great environmental plasticity, with the ability to grow in impoverished or marginal soils

* Corresponding author. Tel.: +56 45 325635; fax: +56 45 325634.
E-mail address: carriaga@ufro.cl (C. Arriagada).

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and to accumulate high quantities of heavy metals (Arriagada et al., 2004). *Eucalyptus* species are able to develop AM symbiosis. The joint inoculation of this plant with AM and saprophytic fungi increased its capacity of accumulation and tolerance to some heavy metals such as Cd, Al and Pb. The inoculation with both fungi also increased the capacity of *Eucalyptus* to remove these heavy metals from contaminated soils (Arriagada, 2001).

Phytoremediation, the use of plants to remove toxic metals from soils, is emerging as a potential strategy for cost-effective and environmentally friendly remediation of contaminated soils (Glass, 2000). Most plants used in phytoremediation of soil contaminated with heavy metals are herbaceous. However, cultivation of non-hyperaccumulating but highly mycorrhizal plants that produce large amounts of biomass on contaminated soil are recommended as a phytoremediation practice.

The aim of this work was to determine whether there were interactions between the AM fungus *Glomus deserticola* and the saprophytic fungi *Trametes versicolor* and *Coriolopsis rigida* and whether this increased the tolerance and accumulation of Zn in *Eucalyptus globulus*.

2. Materials and methods

2.1. In vitro experiments

The effect of Zn on spore germination and hyphal length in *G. deserticola* (Trappe, Bloss and Menge) from the Instituto de Investigaciones Agrobiológicas de Galicia (CSIC) was tested in 9-cm-diameter plastic Petri dishes. Spores of *G. deserticola* were isolated by wet-sieving the soil (Gerdemann, 1955) from alfalfa plant pot cultures; spores were then stored in water at 4 °C. Ten surface-sterilized spores (Mosse, 1962) per plate were placed 1 cm from the edge of a Petri dish with 10 ml of 10 mM 2-(N-morpholin) ethane sulphonic acid (MES) buffer (pH 7) plus 0.04 g of Gel-Gro (ICN Biochemicals, Aurora, OH, USA). $ZnSO_4 \cdot 7H_2O$ was added to Petri dishes to a final concentration of 0, 10, 20, 50, 100 or 200 mg l^{-1}. Ten replicates were used. The plates were incubated at 25 °C in the dark for 21 days and were sealed to reduce dehydration and contamination. Hyphal length of the germinated *G. deserticola* spores was determined under a binocular microscope at 40× magnification at the end of the experiment, using the gridline intersect method (Marsh, 1971). All fungal mycelia were measured. In the 100 and 200 mg l^{-1} Zn treatment conditions, the concentration of Zn was analysed in the Gel-Gro medium after 21 days of AM spore culture (Mingorance, 2002). Gel-Gro medium with 200 mg l^{-1} Zn but without spore culture was used as control. Ten replicates were used in these experiments.

The saprophytic fungi *C. rigida* and *T. versicolor* were isolated using the particle washing method and a multichamber washing apparatus (Widden and Bisset, 1972). These fungi were classified as described by McAllister (1992). Strains are kept at the fungal culture collection of the Facultad de Ciencias Agropecuarias y Forestales, Universidad de La Frontera in Temuco, Chile. Both saprophytic fungi were transferred to tubes of potato dextrose agar (PDA, DIFCO) and 2% malt extract at 4 °C as stock culture. An aqueous suspension in sterile distilled water containing saprophytic fungus mycelia was prepared from cultures grown in PDA for 1 wk at 28 °C. Two millilitres of this suspension were inoculated in 250 ml flasks containing 125 ml of sterile AG liquid medium in a shaker at 28 °C. The AG medium comprised 1 g glucose, 0.4 g asparagine, 0.05 g $MgSO_2$, 0.05 KPO_2 and 100 ml distilled water (Galvagno, 1976). $ZnSO_4 \cdot 7H_2O$ was added to AG medium to a final concentration of 0, 500 and 1000 mg l^{-1} Zn. The culture medium was filtered through a disk of filter paper, dried at 80 °C for 72 h and the dry mycelia of the saprophytic fungi were weighed (McAllister, 1992). In the 500 and 1000 mg l^{-1} Zn treatment conditions, the concentration of Zn was analysed in the AG medium after 2 weeks of culture with *C. rigida* and *T. versicolor* (Mingorance, 2002). AG medium with 500 mg l^{-1} Zn but without fungal culture was used as control. Ten replicates were used in these experiments.

2.2. Greenhouse experiments

The experiments were carried out using eucalyptus (*E. globulus* Labill) as test plants. The seeds were supplied by the Centro de semillas forestales, Departamento de Ciencias Forestales, Universidad de La Frontera (Temuco, Chile). Seeds were surface-sterilized with $HgCl_2$ for 10 min, thoroughly rinsed with sterilized water, and then sown in moistened sand. After germination, uniform seedlings were planted in 0.3 l pots filled with a mixture of sterilized sand:soil at a proportion of 1:1 (V:V). The soil, classified as an Andisol (Acrudoxic Hapludands), with low P content (7.3 mg kg^{-1}, $NaHCO_3$-extractable), is moderately acidic (pH 5.4) with good drainage and water infiltration. The plants were grown in a greenhouse with supplementary light provided by Sylvania incandescent and cool-white lamps, 400 E m^{-2} s^{-1}, 400–700 nm, with a 16/8 h day/night cycle at 25/19 °C and 50% relative humidity. The plants were watered from below and fed every week with 10 ml of a nutrient solution plus 50 mg l^{-1} of P (Hewitt, 1952), which did not affect negatively the AM colonization of *E. globulus* root (Arriagada, 2001).

The *G. deserticola* inoculum was a root-and-soil inoculum consisting of rhizosphere soil containing spores and colonized root fragments of *Medicago sativa* L. Each pot was inoculated with 8 g, an amount determined to achieve high levels of root colonization. Uninoculated plants were given a filtrate (Whatman no. 1 paper) of the inoculum containing the common soil microflora, but free of AM fungal propagules.

Sterilized barleys seeds were used as saprophytic fungal inoculums carriers. The seeds were inoculated with a thin slice of PDA (1 × 1 cm) with mycelia of a 14 days old culture of the saprophytic fungi *C. rigida* or *T. versicolor* grown at 28 °C. Soil pots were inoculated with 10 barleys seeds grown with the saprophytic fungi under static incubation at 28 °C for 20 weeks. Pots with 10 barley seeds but without fungal culture were used as control.

A 6 × 5 full factorial randomized experimental design was used. There were six treatments: (1) uninoculated controls, (2) soil pot inoculated with *C. rigida*, (3) soil pot inoculated with *T. versicolor*, (4) soil pot inoculated with *G. deserticola*, (5) soil pot inoculated with *C. rigida* and *G. deserticola*, and (6) soil pot inoculated with *T. versicolor* and *G. deserticola*. Zn was applied to *E. globulus* pots at the concentration of 0, 10, 100, 500 and 1000 mg Zn kg^{-1} of soil. Five replicate pots per treatment and Zn concentration were used. A total of 150 pots (Six treatments × five Zn doses × five replicates), were used. Plants were inoculated at the time of transplant (After three weeks of growth). The saprophytic fungi were inoculated at the same time as *G. deserticola*.

The plants were harvested after 12 weeks and the dry mass was determined. After the harvest, two fresh weight samples were taken at random from the root system. One of the samples was cleared and stained (Phillips and Hayman, 1970), and the percentage of root length colonization was measured (Giovannetti and Mosse, 1980). In the second sample, succinate dehydrogenase (EC 1.3.99.1) (SDH) activity was measured in fungal mycelia violet stained with the formazan deposits formed by the reduction of tetrazolium salts at the expense of added succinate (MacDonald and Lewis, 1978). The percentage of AM fungal mycelia with SDH activity was determined in 30 root segments per plant that were mounted on slides and examined at ×160 magnification under a compound microscope (Ocampo and Barea, 1985).

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