Applied Soil Ecology 61 (2012) 280–287

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effects of arbuscular mycorrhizal inoculation on metallophyte and agricultural plants growing at increasing copper levels

Sebastián Meier[a], Fernando Borie[a], Gustavo Curaqueo[a], Nanthi Bolan[b], Pablo Cornejo[a,*]

[a] Departamento de Ciencias Químicas y Recursos Naturales, Scientific and Technological Bioresource Nucleus, BIOREN-UFRO, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile
[b] Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes Campus, SA 5095, Australia

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form
15 September 2011
Accepted 28 October 2011

Keywords:
Copper contaminated soils
Cu-adapted AM fungi
Glomalin-related soil protein
Phytoremediation

ABSTRACT

A pot culture experiment was carried out to assay the behavior of different arbuscular mycorrhizal (AM) fungal inocula on plant growth and copper (Cu) uptake using two metallophytes (*Oenothera picensis* and *Imperata condensata*) and one agricultural plant (*Helianthus annuus*) grown at increasing Cu supply levels. Plants were established in a Cu polluted soil spiked with 0, 150, 300 or 450 mg Cu kg^{-1}, and inoculated or not with: (i) Cu-adapted AM fungi (GA) or (ii) the Cu non-adapted strain *Glomus claroideum* (GC). Differences in plant biomass between inoculated and uninoculated plants were found, which were dependent on the AM fungal inocula used and the Cu level applied. Although the beneficial effect of AM fungi in promoting plant biomass production was not observed in metallophytes plants, a positive interaction between GA and *H. annuus* increased the shoot growth, especially at higher Cu levels. In addition, the Cu transfer from the roots to the shoots was low, remaining mostly at root level, especially in non-mycorrhizal plants; however AM fungi produced changes in Cu distribution increasing the translocation to the shoots. Differences in AM fungal parameters (root colonization, spore number and glomalin production) were strictly dependent on the Cu level and the AM fungal inoculum, suggesting the existence of certain compatibility, which was dependent on the particular combination AM-plant used. Specifically, the glomalin accumulation and Cu-bound to glomalin were significantly higher in AM colonized *H. annuus* plants, which could suggest a highly efficient way to reduce the Cu toxicity levels in soil. Therefore the use of *H. annuus* with AM fungal could promote phytostabilization processes.

1. Introduction

Copper (Cu) is an essential trace element for normal plant growth and development. However, an excessive amount of this element in soil is highly toxic to both higher plants and microorganisms, often resulting in vegetation degradation, soil quality decrease and, as a consequence, the normal functioning of the ecosystem is affected (Adriano, 2001; Bolan et al., 2003; Wong, 2003). Several environmental remediation systems involving physical, chemical, or biological treatments have been developed for reclamation of metal contaminated soils in the last decades (Mulligan et al., 2001). However, these treatments are expensive, and alter the soil's physicochemical and biological properties and therefore are considered environmental unfriendly.

Recently, the potential role of higher plants in remediation of metal-polluted soils has acquired relevance (Pilon-Smits, 2005). The use of vegetation for landscaping, stabilization and pollution control is probably the most realistic approach to the reclamation of the land impacted by high metal concentrations (Robinson et al., 2007; Bolan et al., 2011). Nevertheless, an important factor that determines the successful vegetation in metal polluted sites is the initial plant establishment, which is often limited by metal toxicity, low nutrient contents and poor soil physical structure (Ye et al., 2002). For the above reasons, the long-term success of phytoremediation programs in metal contaminated soils has been limited. Among factors involved, the lack of knowledge about the role of microbial communities could explain some failures.

Soil microorganisms are involved in diverse biochemical processes, such as soil formation, energy transfer and nutrient cycling, which enhance and accelerate vegetation processes and thereby increase the stability of polluted ecosystems (Moynahan et al., 2002). However, managing soil microorganisms in phytoremediation must include the use of those forming symbiotic associations with plant roots such as the arbuscular mycorrhizal (AM) fungi as prerequisite for any soil restoration program to be successful (Haselwandter and Bowen, 1996; Meier et al., 2011).

It is well known that AM fungi improve plant establishment in metal polluted soils, and even, some studies concluded that the symbiosis is partly responsible for plant survival in those extreme environments (Carvalho et al., 2006; Hildebrandt et al.,

* Corresponding author. Tel.: +56 45 325433; fax: +56 45 325440.
E-mail address: pcornejo@ufro.cl (P. Cornejo).

0929-1393/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2011.10.018

2007; Meier et al., 2012). In this sense, AM fungal colonization contributes to enhancement of plant establishment, through improving plant nutrition, particularly phosphate and some trace elements (Reinhardt, 2007; Meier et al., 2011). In addition, AM fungi can improve soil structure through the combined actions of network of external mycelium and the production of a glycoprotein known as glomalin, which also has the capacity to sequester heavy metals from soil (González-Chávez et al., 2004; Cornejo et al., 2008). Therefore, plants proposed for phytoremediation might require the use and selection of the most effective AM fungi in order to survive in such constraint conditions. This selection should be supported by the knowledge of metal-tolerant fungal species able to grow and function on polluted as well as adapted to nutrient impoverished soils (del Val et al., 1999). In order to understand the interactions among metals, AM fungi and higher plants, it is necessary to study and compare the diversity of AM fungi associated with metal-tolerant and non-tolerant plants in metal polluted and unpolluted soils for making a selection of those which are suitable for phytoremediation purposes; however, at present only few studies have been reported on this aspect (Vivas et al., 2006).

Oenothera picensis and *Imperata condensata* are Cu metallophyte plants naturally growing in metal polluted soils at Central Chile. These plants have been known to tolerate Cu toxicity, thereby offering a potential to be used in phytoremediation programs (Ginocchio, 2000; Cornejo et al., 2008; González et al., 2008). Some studies have investigated the Cu tolerance of these plants (González et al., 2008; Gaete et al., 2010); however, few of them have been focused on the role of their rhizosphere associations, especially with AM fungi. Recent studies have demonstrated the positive role of the indigenous AM fungi in promoting metallophyte establishment in Cu polluted soils (Meier et al., 2011). Nevertheless, there are no reports comparing the effectiveness of different AM fungal strains, either isolated from Cu contaminated soils or from non-polluted areas enhancing plant establishment of both Cu tolerant and non-metal adapted plants.

Therefore, the aim of this research was to compare the behavior of an indigenous Cu tolerant mycorrhizal fungal (GA) mix versus a non-metal adapted AM fungus strain (*Glomus claroideum* – GC) using two Cu metallophytes and one agricultural plant, all growing at increasing Cu supply levels. The effectiveness of AM fungal inoculation was tested through the analysis of plant biomass production, Cu concentration in plant tissues and AM development parameters, including glomalin accumulation and Cu sequestration by this glycoprotein.

2. Materials and methods

2.1. Experimental design

The soil used in this study corresponded to a Cu contaminated soil (830 mg total Cu kg^{-1} soil and 330 mg DTPA extractable Cu kg^{-1} soil; Cornejo et al., 2008) obtained near to Ventanas Cu smelter (CODELCO), in the Puchuncaví Valley, Central Chile (32°46′30″S, 71°28′17″W). The Cu contaminated soil was spiked with four additional Cu levels: 0, 150, 300 or 450 mg Cu kg^{-1} (applied as $CuCl_2{\cdot}2H_2O$, Sigma reagent). In addition, there were three plant species and three AM fungi treatments, which included: (i) a mixture of autochthonous Glomeromycota (GA) fungi, (ii) a strain of *G. claroideum* (GC), and (iii) uninoculated plants. A completely randomized design with three replicates per each combination was used, with a total of 108 experimental units.

2.2. Plant species

Two metallophytes, *O. picensis* (formerly named *O. affinis*) and *I. condensata* (Poaceae) were used. Both plants have been described as Cu tolerant species (González et al., 2008; Cornejo et al., 2008). Seeds of *O. picensis* and stolons of *I. condensata* were collected *in situ* from Cu-polluted areas to produce plantlets. The plant collection area was a Mediterranean ecosystem strongly affected by the deposit of metal-rich particles, located approximately at 1.5 km southeast from the Ventanas Cu smelter (CODELCO), at the Puchuncaví Valley, Central Chile (32°46′30″S, 71°28′17″W). In addition, commercial seeds of the agricultural plant, sunflower (*Helianthus annuus*) was included due to its potential ability to accumulate Cu without being overly sensitive to Cu toxicity (Lin et al., 2003).

2.3. Soil characteristics and plant growth conditions

The soil used in this study belongs to Chilicauquén series, fine sandy loam in texture, moderately deep, formed on a substrate of sandstone cemented with clay from the upper horizons. This soil was sieved through a 2 mm mesh and diluted with quartz-sand (<1 mm) (2:1 soil:sand, v/v), sterilized by tyndallization for three consecutive days, and air dried. Soil mixture was placed in 500 mL pots. After sterilization, the soil/sand mixture was treated with 0, 150, 300 or 450 mg Cu kg^{-1} soil by adding known amounts of $CuCl_2{\cdot}2H_2O$ solution, then left to equilibrate for two weeks.

Seeds of *O. picensis* and *H. annuus*, and stolons of *I. condensata* were surface sterilized with 2% Cloramin-T solution for 5 min and rinsed thoroughly. The seeds and stolons were grown under greenhouse conditions (25 ± 3 °C/15 ± 3 °C day/night temperatures; 16/8 h light/dark photoperiod; 80–90% relative humidity) for 4 weeks using a sepiolite:quartz sand:vermiculite (1:1:1, v/v/v) mix as substrate before transplanting. At transplanting, the plants were or not AM fungal inoculated. The Cu-adapted indigenous AM fungi (GA) were isolated by wet sieving and decanting (Gerdemann and Nicolson, 1963) from rhizosphere soil of *O. picensis* and *I. condensata* plants growing in Cu polluted soils of Puchuncaví Valley (Central Chile), mixed and transferred to an open pot culture using sepiolite:quartz:vermiculite (1:1:1, v/v/v) mix as substrate. *O. picensis* and *Plantago lanceolata* were used as host plants. After 6 months of plant growth, shoots were eliminated and the substrate containing about 250 spores per 100 g, 3.0 m of AM hyphae per g, and fragments of colonized roots was used as GA inoculum. A preliminary morphological analysis revealed that almost all the spores present in the inocula belonged to *Glomus* genus, being *Glomus* aff. *intraradices* the dominant ecotype. Other present ecotypes were *Acaulospora* aff. *lacunosa*, *Entrophospora infrequens* and others, which belongs to *Gigaspora* and *Scutellospora* genus, but such ecotypes were found in a very low density and only in some inoculum samples. *G. claroideum* (GC) strain was isolated from agricultural soils agricultural soils near Temuco city (Southern Chile) and used as a reference of non-Cu adapted AM fungi. The GC inoculum was obtained in similar way that for GA, but using *Sorghum bicolor* and *Trifolium repens* as host plants. In both cases, a mixture of rhizosphere substrate containing spores (about 300 spores per 100 g), hyphae (about 4 m per g) and mycorrhizal root fragments was used as inoculum. 10 g of each inoculum were added to the respective pots just below the seedlings. Uninoculated plants (NM) received an equivalent amount of autoclaved inoculum. Plants were grown for 3 months under greenhouse conditions and then harvested.

2.4. Plant and AM fungi analysis

At harvest, shoots and roots were separated, dried at 70 °C for 2 days and weighed. Then, the samples were ground, ashed at 550 °C and digested using a $H_2O/HCl/HNO_3$ mixture (8/1/1, v/v/v). The