Available online at www.sciencedirect.com
ScienceDirect
Forest Ecology and Management 241 (2007) 200–208
Forest Ecology and Management
www.elsevier.com/locate/foreco

Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochtonous arbuscular mycorrhizal fungi

L. Ouahmane [a,b], J. Thioulouse [c], M. Hafidi [a], Y. Prin [d], M. Ducousso [d], A. Galiana [d], C. Plenchette [e], M. Kisa [f], R. Duponnois [f,*]

[a] *Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, Morocco*
[b] *Direction Régionale des Eaux et Forêts du Haut Atlas, Marrakech, Morocco*
[c] *CNRS, Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université Claude Bernard Lyon 1, France*
[d] *CIRAD, UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), Montpellier, France*
[e] *INRA, UMR BGA, Dijon, France*
[f] *IRD, UMR 113 CIRAD/INRA/IRD/AGRO-M/UM2, Laboratoire des Symbioses Tropicales et Méditerranéennes (LSTM), Montpellier, France*

Received 27 May 2006; received in revised form 8 December 2006; accepted 9 January 2007

Abstract

The potential benefits of inoculation with AM (arbuscular mycorrhizal) fungi were investigated on Atlas Cypress (*Cupressus atlantica* G.), an endemic Cupressacea in Morocco. The parameters under study were (i) the growth of the plant, (ii) the functional diversity of soil microflora and (iii) the rock phosphate (RP) solubilizing activity. *C. atlantica* growth was measured after 12 months of culture in plastic bags arranged in a randomised complete block design with 10 replicates per treatment. Fungal inoculation consisted of either *Glomus intraradices* alone or a mixture of native AM fungi. P amendment was supplied under the form of Khouribga Rock Phosphate (KRP) powder. Microbial catabolic diversity was assessed by measuring CO_2 production of SIR (substrate induced respiration) responses. Results showed that: (i) the fungal symbionts were effective to improve the growth of *C. atlantica*, confirming the requirement of mycorrhizal symbiosis for the successful establishment of *C. atlantica* in a degraded soil; (ii) *G. intraradices* appeared to be the most effective in promoting growth of *C. atlantica*, whereas indigenous AM fungi were relatively ineffective. Native AM fungi inoculation strongly modified functional abilities of the soil microflora, and in the treatments with P amendment, growth stimulations of native AM fungi inoculation were significantly higher than those of *G. intraradices* inoculation for the shoot growth and leaf P content; (iii) *C. atlantica* plants inoculated with native AM fungi could mobilize P from KRP more efficiently than those mycorrhized with *G. intraradices*. A strong interaction between KRP amendment and fungus inoculation was detected for the leaf P content results. In conclusion, the use of a mixture of native AM fungi combination may increase the chance of including one very effective fungal isolate, but also, creates a more favourable environment for the development of ecosystems processes.

Keywords: Arbuscular mycorrhizas; Diversity; Soil functional abilities; Rock phosphate; Morocco

1. Introduction

Mediterranean ecosystems are subjected to disturbances following scarce and irregular rainfall, long dry and hot summers and man-mediated degradative activities (overgrazing, non-regulated cultivation techniques, deforestation, etc.). Such degraded ecosystems are usually characterised by a disturbed vegetation cover accompanied by a rapid erosion of surface soil (Herrera et al., 1993). The desertification process involves a loss or reduction of major physicochemical and biological soil properties (Requena et al., 2001) and significantly reduced arbuscular mycorrhizal (AM) soil inoculum potential (Jasper et al., 1989, 1991; Brundrett, 1991; Herrera et al., 1993; Duponnois et al., 2001a). AM fungi

* Corresponding author. Present address: IRD, Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386, Dakar, Senegal. Tel.: +221 849 33 22; fax: +221 849 33 02.
E-mail address: robin.duponnois@ird.sn (R. Duponnois).

0378-1127/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.foreco.2007.01.015

have been found to be essential components of sustainable soil-plant systems (Smith and Read, 1997; van der Hejden et al., 1998; Schreiner et al., 2003) and particularly important in counteracting desertification of Mediterranean ecosystems (Carpenter and Allen, 1988; Brundrett, 1991). They increase plant uptake of phosphorus (Duponnois et al., 2005), micronutrients (Bürkert and Robson, 1994) and nitrogen (Barea et al., 1991). They enhance water absorption (George et al., 1992) and act as antagonists against some plant pathogens (Dehne, 1982; Lendzemo et al., 2005). AM fungal symbiosis changes root functions (i.e. root exudation) (Graham et al., 1981; Marshner et al., 1997), modifies carbohydrate metabolism of the host plant (Shachar-Hill et al., 1995) and interacts with rhizosphere populations (Hayman, 1983; Azaizeh et al., 1995; Andrade et al., 1997, 1998). The structure and functionalities of these AM associated microbial communities differ from those of the rhizosphere (Duponnois et al., 2005) and this microbial compartment has been named ''mycorrhizosphere'' (Linderman, 1988).

Plant mineral nutrition depends mainly on the phosphorus content of soil, which can be assimilated only as soluble phosphate. Hence the use of rock phosphate (RP) as a fertilizer for P-deficient soils has received significant interest in recent years since they are natural, inexpensive and available fertilizers. However their solubilization rarely occurs in non-acidic soils (Caravaca et al., 2004, 2005a). Physical and chemical weathering of mineral phosphates is mainly realised along plant roots in the rhizosphere. This part of soil supports large microbial communities that facilitate weathering of minerals by producing organic acids, phenolic compounds, protons and siderophores (Drever and Vance, 1994; Landeweert et al., 2001). Among microbial groups that could solubilize mineral phosphates and improve plant phosphorus nutrition are AM fungi. Arbuscular mycorrhizal fungal inoculation induced spectacular stimulations of the plant growth and P foliar content (Guissou et al., 2001; Caravaca et al., 2004, 2005a,b; Duponnois et al., 2005).

It is well known that AM fungal inoculum potential is very low in degraded Mediterranean ecosystems and an increase of this fungal inoculum potential is needed in both natural and artificial revegetation processes (McGee, 1989). It has already been shown that AM inoculation of plants is very efficient in establishing plants on disturbed soils (Estaun et al., 1997; Duponnois et al., 2001b). There are two main approaches in order to increase and maintain high populations of infective AM propagules in soil: (i) screening of AM fungal isolates (native or exotic isolates) for their effect on the plant growth under controlled conditions and an inoculation of the soil with the most efficient AM strains and (ii) adoption of field practices to manage and improve the inoculum potential of indigenous mycorrhizae. In forestry, the inoculation practice is generally used in tree nurseries in order to help tree establishment (Plenchette, 2000), but also to improve the quality of the planted soil (Franson and Bethlenfalvay, 1989). In a previous study, controlled mycorrhization with an allochtonous AM fungus (*Glomus intraradices*) has significantly improved the growth of *Cupressus atlantica* and strongly modified soil microbial functionalities (Ouahmane et al., 2006). Although it has been shown that native AM fungi are important contributors to ecosystem productivity and functioning (van der Hejden et al., 1998; Requena et al., 2001; Alguacil et al., 2005; Caravaca et al., 2005b), little is known about the potential impact of native AM fungi on the host plant mycorrhizal dependency, microbial soil biofunctioning and subsequent biological processes (i.e. RP weathering).

The main objectives of this investigation were to assess how inoculation with a mixture of native AM fungi or a single AM inoculation with *G. intraradices* affect (i) the growth of Atlas Cypress (*C. atlantica* G.), an endemic Cupressacea of Morocco, (ii) the functional diversity of soil microflora and to determine (iii) if microbial activity increased P uptake from RP under the tested conditions.

2. Materials and methods

2.1. Preparation of fungal inoculums

Soil samples were collected from the rhizosphere of *C. atlantica* at the Idni station (8°17′02″W, 31°54′34″, 1700 m above sea level) located in the N'Fis valley (Haut Atlas, Morocco). They were taken from 10 individual trees, 2 m from the trunk under the canopy. Each sample consisted of five 100 g sub-samples collected at the 20 cm depth. The soil was carefully mixed and spores of AM fungi were extracted from each sub-sample (100 g) by wet sieving and decanting, followed by sucrose centrifugation (Sieverding, 1991). The supernatant was poured through a 50 μm sieve and rinsed with tap water. Fungal spores were surface sterilized with a solution of chloramine T (0.2 g l^{-1}) and streptomycine (0.2 g l^{-1}) (Mosse, 1973) in order to eliminate the mycorrhizosphere microflora. Then they were kept in distilled water at 4 °C for 2 days before use.

The AM fungus *G. intraradices* Schenk & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was multiplied on leek (*Allium porrum* L.) on Terragreen™ substrate. This culture substrate (calcined clay, average particle size 5 mm) used for propagation of AM fungi is an attapulgite from Georgia (Plenchette et al., 1996). After 12 weeks under greenhouse conditions, leek plants were uprooted, gently washed and roots cut into 0.5 cm long pieces bearing around 250 vesicles cm^{-1}.

Seeds of maize (*Zea maize* L.) were surface-sterilized with 1% NaOCl for 15 min and rinsed with distilled water. They were pre-germinated for 2 days in Petri dishes on humid filter paper at 25 °C in the dark. The germinating seeds were used when rootlets were 1–2 cm long.

The soil was collected under *C. atlantica* in High Atlas Mountains (Morocco), crushed, passed through a 2 mm sieve and autoclaved (140 °C, 40 min) to eliminate native micro-organisms. After autoclaving, its chemical characteristics were as follows: pH (H_2O) 7.7; clay (%) 4.4; fine silt (%) 28.6; coarse silt (%) 12.6; fine sand (%) 27.9; coarse sand (%) 19.45; carbon (%) 3.15; nitrogen (%) 0.14; C/N 23; P (Olsen) 13.1 mg kg^{-1}. Black plastic bags (1 dm^3) were filled with this autoclaved soil.