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ORIGINAL ARTICLE

# Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.)

**Fatma Tajini [a,b,c,*], Mustapha Trabelsi [b], Jean-Jacques Drevon [a]**

[a] *INRA, UMR Eco&Sols, Place Viala, 34060 Montpellier Cedex 01, France*
[b] *Ecole Supérieure d'Agriculture de Mateur, Bizerte 7030, Tunisia*
[c] *Faculté des Sciences de Gafsa, 2112 Sidi Ahmed Zarroug, Tunisia*

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**Abstract** This study compared the response of common bean (*Phaseolus vulgaris* L.) to arbuscular mycorrhizal fungi (AMF) and rhizobia strain inoculation. Two common bean genotypes i.e. CocoT and Flamingo varying in their effectiveness for nitrogen fixation were inoculated with *Glomus intraradices* and *Rhizobium tropici* CIAT899, and grown for 50 days in soil–sand substrate in glasshouse conditions. Inoculation of common bean plants with the AM fungi resulted in a significant increase in nodulation compared to plants without inoculation. The combined inoculation of AM fungi and rhizobia significantly increased various plant growth parameters compared to simple inoculated plants. In addition, the combined inoculation of AM fungi and rhizobia resulted in significantly higher nitrogen and phosphorus accumulation in the shoots of common bean plants and improved phosphorus use efficiency compared with their controls, which were not dually inoculated. It is

* Corresponding author at: Faculté des Sciences de Gafsa, 2112 Sidi Ahmed Zarroug, Tunisia. Tel.: +216 94120093.
E-mail addresses: fatmatajini@yahoo.fr (F. Tajini), mus.trabelsi@yahoo.fr (M. Trabelsi), drevonjj@supagro.inra.fr (J.-J. Drevon).



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concluded that inoculation with rhizobia and arbuscular mycorrhizal fungi could improve the efficiency in phosphorus use for symbiotic nitrogen fixation especially under phosphorus deficiency.



## 1. Introduction

Phosphorus and nitrogen constitute the most limited nutriment for vegetative growth. In order to assess the capacity of plant to acquire nutrients, arbuscular mycorrhizal fungi and rhizobia are two of the most important plant symbionts. They play a key role in natural ecosystems and influence plant productivity, plant nutrition and improved inhibition of fungal plant pathogens (Demir and Akkopru, 2007). Mycorrhiza benefits the host through mobilization of phosphorus from non-labile sources, whereas rhizobia fixes $N_2$ (Scheublin and Vander Heijden, 2006). Previous works on the tripartite symbiosis legume–mycorrhiza–rhizobia have shown stimulatory (Edwards et al., 1998; Xiao et al., 2010) or inhibitory (Söderberg et al., 2002; Scheublin and Vander Heijden, 2006; Franzini et al., 2010) effects on each other or on the growth of plants.

A few studies have shown that some bacterial species respond to the presence of certain AM fungi (Andrade et al., 1997; Artursson et al., 2006), suggesting a high degree of specificity between bacteria associated with AM fungi. Thus, the specific bacteria together with AM fungi may create more indirect synergism for plant growth (Barea, 1997), including nutrient acquisition (Barea et al., 2002) and enhancement of root branching (Gamalero et al., 2004). In addition, the AM fungi themselves have also been shown to have an impact on the composition of bacterial communities in their mycelium environment (Artursson et al., 2006).

On the other hand Aysan and Demir (2009) reported that the information on the mechanisms controlling interactions of bacteria with AM fungi and plant roots in the mycorrhizosphere and their activities are very difficult to generalize because the interactions involving arbuscular mycorrhiza, root rot fungi and *Rhizobium* vary with the microbial species and plant cultivars.

In this paper, we focus on interactions between rhizobia and AM fungi with common bean in their influence on plant growth and investigate whether sensitivity of symbiotic nitrogen fixation to phosphorus deficiency was restored by symbiosis with arbuscular mycorrhizal fungi in soil condition.

## 2. Material and methods

Two common bean (*Phaseolus vulgaris* L.) genotypes (CocoT and Flamingo) were used in this study grown in sand–soil culture. The common bean genotypes were inoculated or not (controls) with AMF and in both cases received similar rhizobial inoculation (see below).

### 2.1. Biological material

The common bean genotypes were CocoT and Flamingo. The first one was selected as a pure line from the local cultivar Coco whereas Flamingo was selected on the basis of its tolerance to salinity (Jebara et al., 2001) among a collection initially supplied by B. Voyesset from CIAT (Colombia). Seeds were surface-sterilized with 1.3% calcium hypochloride for 15 min with constant stirring, and subsequently washed with sterile distilled water. They were germinated on 0.8% sterile agar plates for 3 days at 28 °C in the dark, with a germination rate of 80%. Rhizobial inoculation was performed by soaking the seedlings of common bean for 45 min within a freshly prepared suspension of *Rhizobium tropici* CIAT899 containing $10^8$ bacteria $ml^{-1}$.

Thereafter the seedlings were grown in 1000 ml pots filled with autoclaved sand–soil mixture (9:1 v:v) recolonized with soil bacteria according to Jansa et al. (2002). This potting mixture was inoculated with AMF or a non-mycorrhizal inoculum (Control was not inoculated with *Glomus intraradices*). By placing the bean seedlings in the potting mixture, they became inoculated with *G. intraradices* BEG157 (Schenck & Smith) or with the non-mycorrhizal mixture (as a control). The inoculum used for the pots consisted of chopped roots of pot cultures planted with leek (*Allium porrum*) and grown for 18 months in a glasshouse. Fifty grams of AMF inoculum was thoroughly mixed into each pot that received approximately 1000 spores of the AMF species contained at least 20 infective propagules of AMF per gram of chopped root.

The amount of mycorrhizae substrate was characterized by low available N (0.007%) and P (0.001%). In non mycorrhizal treatments, each pot filled with same amount of mycorrhizae free substrate.

### 2.2. Growth conditions

Trials were performed in a temperature-controlled glasshouse with night/day temperatures of 25/35 °C, and a 16 h photoperiod with complementary illumination of 400 μmol photons $m^{-2}\ s^{-1}$. Seedlings inoculated with *R. tropici* CIAT899 were grown in soil–sand substrate with or without *G. intraradices*.

Pots were watered with distilled water every 2 days until harvest, and received once a week the Vadez et al. (1996) nutrient solution: macroelements: $K_2SO_4$ (1.25 mM), $MgSO_4{\cdot}7H_2O$ (2.05 mM), $CaCl_2$ (3.3 mM); microelements: Fe EDDHA (8.5 μM Fe as sequestrene), $H_3BO_3$ (4.0 μM), $MnSO_4$ (6.0 μM), $ZnSO_4$ (0.9 μM), $CuSO_4$ (1.0 μM), $NaMoO_4$ (0.1 μM). The nutrient solution was supplemented with 2 mmol urea $plant^{-1}$ during first two weeks, 1 mmol urea $plant^{-1}$ during the next two weeks and no more urea during the last two weeks.

The pots were distributed in a complete randomized block design with 6 replications and one plant only per pot.

### 2.3. Assessment of AMF colonization

The plants were harvested after 50 days of growth. Half of the root system was used for estimation of the extent of root colonization by the AMF as follows: roots were cleared in KOH 10% (w:v) at 80 °C for 30 min followed by rinsing with water and two rinses with 1% HCl during 1 h. Thereafter, the roots were immersed at 80 °C for 1.5 h in the staining solution