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Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil

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ABSTRACT

In sustainable agriculture, arbuscular mycorrhizal (AM) fungal inoculation in agronomical management might be very important, especially when the efficiency of native inocula is poor. Here, we assessed the effect of native and exotic selected AM fungal inocula on plant growth and nutrient uptake in a low input *Trifolium alexandrinum–Zea mays* crop rotation. We evaluated the effects of four exotic AM fungal isolates on *T. alexandrinum* physiological traits in greenhouse. Then, the field performances of *T. alexandrinum* inoculated with the exotic AMF, both single and mixed, were compared to those obtained with a native inoculum, using a multivariate analysis approach. Finally, we tested the residual effect of AM fungal field inoculation on maize as following crop. Multivariate analysis showed that the field AM fungal inoculation increased *T. alexandrinum* and *Z. mays* productivity and quality and that the native inoculum was as effective as, or more effective than, exotic AM fungal isolates. Moreover, the beneficial effects of AMF were persistent until the second year after inoculation. The use of native AMF, produced on farm with mycotrophic plants species, may represent a convenient alternative to commercial AM fungal inocula, and may offer economically and ecologically important advantages in sustainable or organic cropping systems.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are beneficial microbes fundamental for soil fertility of natural and agricultural ecosystems (Smith and Read, 2008). They colonize the roots of most land plant species, including crop, pasture and horticultural plants. AM fungi (AMF) increase plant growth and nutrient uptake, and improve plant tolerance to root pathogens and drought (Augé, 2001; Graham, 2001; Smith and Read, 2008). Because of the key role of AMF on plant health and fitness, the productivity of agro-ecosystems is affected by their abundance and diversity in the soil (Lekberg and Koide, 2005). In sustainable agricultural systems, the implementation of AM fungal inoculation in agronomical management is important when mycorrhizal potential of native soil is inadequate, as to quantity and/or quality (Requena et al., 1996; Koide and Mosse, 2004).

In field experiments, a higher mycorrhizal colonization due to AM fungal inoculation was positively correlated with crop yields and P uptake, which were increased by more than 30% (McGonigle, 1988; Lekberg and Koide, 2005). However, responses of plants to AM fungal inoculation were reported to depend on physical and chemical soil characteristics (Davis et al., 1983; George, 2000), native mycorrhizal populations (Requena et al., 2001), functional differences among isolates (Jakobsen et al., 1992a; Smith et al., 2000; Munkvold et al., 2004; Avio et al., 2006) and host plants (Streitwolf-Engel et al., 2001; van der Heijden and Sanders, 2002; Klironomos, 2003).

So far, most field studies evaluated plant responses to single AM fungal strains, while only few reports showed the effects of mixed inocula, usually represented by exotic isolates (Clarke and Mosse, 1981; Edathil et al., 1996; Meyer et al., 2005). Such mixed inocula were utilised on the basis of their potential functional complementarity (Koide, 2000). By contrast, the efficiency of mixed native inocula, compared with exotic ones, was poorly studied in agro- and natural ecosystems (White et al., 2008; Requena et al., 2001; Caravaca et al., 2005).

In sustainable agriculture, the use of legume plants in crop rotations is pivotal for the maintenance of soil fertility, due to the tripartite symbiotic interaction between legumes, rhizobia and

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AMF, which affect P and N uptake and N fixation (Rao et al., 1986; Azcón-Aguilar and Barea, 1981; Cleveland et al., 1999; Scheublin et al., 2007).

In this study we assessed the effect of native and exotic selected AM fungal inocula on plant growth and nutrient uptake in a low input, 2 years *Trifolium alexandrinum–Zea mays* crop rotation. First, we evaluated the effects of four exotic AM fungal isolates on *T. alexandrinum* physiological traits in greenhouse. Second, the field performances of *T. alexandrinum* inoculated with the exotic AMF, both single and mixed, were compared with those obtained with a native inoculum, using a multivariate analysis approach. Finally, we tested the residual effect of AM fungal field inoculation on the growth and nutrient uptake of maize as following crop.

2. Materials and methods

2.1. Experiment 1: evaluation of plant physiological traits as affected by four exotic AMF inoculated on T. alexandrinum

2.1.1. Fungal and plant material

The AMF used were: *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd & Trappe 1974, isolate IMA1 from UK (collector B. Mosse) and isolate AZ225C from USA (collector J.C. Stutz), and *Glomus intraradices* N.C. Schenck & G.S. Sm. 1982, isolate IMA5 from Italy (collector M. Giovannetti) and isolate IMA6 from France (collector V. Gianinazzi-Pearson). They were obtained from pot cultures maintained in the collection of the Department of Crop Plant Biology, University of Pisa, Italy. The plant species used was the forage legume *T. alexandrinum* L. cv. Tigri.

2.1.2. Experimental setup

Seeds (20) of *T. alexandrinum* were sown in 600 mL plastic pots containing a mixture (1:1 by volume) of soil and Terragreen (calcinated clay, OILDRI, Chicago, IL, USA). The soil was a sandy loam collected at the Rottaia Experimental Centre, University of Pisa, Italy. Chemical and physical characteristics of the soil used were as follows: pH(H_2O), 8.0; clay, 15.3%; silt, 30.1%; sand, 54.5%; organic matter, 2.2% (Walkley–Black); total N, 1.3‰ (Kjeldahl); total P, 469.5 mg kg^{-1} (Olsen); extractable P, 17.6 mg kg^{-1} (Olsen); extractable K, 149.6 mg kg^{-1}. The mixture was steam-sterilized (121 °C for 25 min, on two consecutive days) to kill naturally occurring AMF. Pots were inoculated either with 90 mL of crude inoculum (mycorrhizal roots and soil containing spores and extraradical mycelium) of one of the four AM fungal isolates or with 90 mL of a sterilized mixture of them (non-mycorrhizal control). In this way, potential differences in AM fungal colonization ability of the four isolates were balanced using such high amounts of inoculum (15% by volume). All the pots received 120 mL of a filtrate, obtained using a mixture of the four crude inocula and of a sample of agricultural soil from a *T. alexandrinum* field, to ensure a common microbiota to all treatments. After emergence, seedlings of *T. alexandrinum* were thinned to 10 to reproduce the usual field densities. Plants were grown in greenhouse, supplied with tap water as needed and with a weekly fertilization of half-strength Hoagland's solution (10 mL per pot). The experiment was a completely randomized design with five treatments (four fungal isolate and the control), five replicates and one harvest. Three months after emergence, plant shoots were harvested by cutting 1 cm above soil level.

2.1.3. Plant growth response and nutrient uptake

At harvest, stems and leaves of *T. alexandrinum* plants were separated and dry weights determined after drying at 95 °C for 48 h. Root systems were removed and dry weights determined on a subsample (half of each root system). Aliquots of dry shoots and roots were used to assess their nutritional status. Percentage of AM colonization was assessed after clearing and staining using lactic acid instead of phenol (Phillips and Hayman, 1970), using the gridline intersect method (Giovannetti and Mosse, 1980).

Tissue N concentrations of shoots were assessed using the Kjeldahl method (Jones et al., 1991). P concentrations of shoots and roots were measured after sulphuric/perchloric acid digestion using the photometric method (Jones et al., 1991). The total N and P contents were calculated by multiplying N and P concentration values by dry weights.

2.2. Experiment 2: field evaluation of growth response and nutrient uptake of T. alexandrinum inoculated with exotic and native AM fungal inocula

This field experiment aimed at evaluating growth and nutritional responses of *T. alexandrinum* inoculated with single and mixed inocula of the four *Glomus* isolates tested in Experiment 1 and with a native AM fungal inoculum.

2.2.1. Fungal and plant material

The fungal material used was: a mixed inoculum (EMix) of the exotic AM fungal isolates tested in Experiment 1; the single inocula of each isolate (IMA1; AZ225C; IMA5; IMA6) and a native inoculum (NMix) containing a mixed fungal population from the Rottaia field site. The AM fungal population of such field site was represented by: *Acaulospora rugosa*, *Acaulospora scrobiculata*, *Acaulospora spinosa*, *Diversispora spurca*, *Glomus clarum*, *Glomus coronatum*, *Glomus etunicatum*, *Glomus geosporum*, *G. intraradices*, *G. mosseae*, *Glomus* spp., *Glomus viscosum*, *Scutellospora aurigloba*, *Scutellospora calospora* (Pellegrino, 2007). The plant species used was the forage legume *T. alexandrinum* cv. Tigri.

2.2.2. Experimental field site

The experiment was settled at the Rottaia Experimental Centre of the University of Pisa, Italy (43°30′86″N–10°19′00″E). The soil was a sandy loam. Chemical and physical characteristics of the soil were: pH(H_2O), 8.4; clay, 9.6%; silt, 23.9%; sand, 66.5%; organic matter, 1.5% (Walkley–Black); total N, 0.7‰ (Kjeldahl); total P, 461.9 mg kg^{-1} (Olsen); extractable P, 14.6 mg kg^{-1} (Olsen). Climatic conditions, typical of Mediterranean areas, were: mean monthly air temperature from 11 °C in February to 30 °C in August; rainfall concentrated in autumn (October–November) and spring (March–April). In the latest 20 years, annual rainfall ranged from 550 to 1180 mm, with a mean of 948 mm.

2.2.3. AM fungal inoculum production

In order to prepare the amount of inoculum needed in the field, AM fungal material was produced in 18 L pots, filled with sandy loam soil and Terragreen (1:1 by volume; see Experiment 1 for physical and chemical characteristics). The substrate was steam-sterilized (121 °C for 25 min, on two consecutive days), to kill naturally occurring AMF. Each exotic single inoculum (IMA1, AZ225C, IMA5, IMA6) was produced in seven pots inoculated with 500 g of crude inoculum, originated from the collection of the Department of Crop Plant Biology. The exotic mixed inoculum (EMix) was obtained by mixing, at the end of inoculum production, equal quantities of each exotic AM fungal isolate. The native inoculum (NMix) was produced in seven pots inoculated with 500 g of soil from the Rottaia field site. In addition, seven 18 L pots were set up by mixing the substrate with 500 g of a sterilized mixture of equal quantities of crude exotic inocula and Rottaia soil, in order to inoculate control plots. *Z. mays* was used as host plant (10 plants per each pot). All pots received 1.5 L of a filtrate, obtained using a mixture of the fungal inocula (see Experiment 1). Pots were