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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

supplied with 2.5 L of deionised water (DW) (irrigation cycle: 4 days) replaced by half-strength Hoagland's solution after two months' growth (2.5 L per pot). After 4 months' growth, maize plants were harvested, the soil and the root systems removed from the pots and air dried. Then, the roots were cut, carefully mixed with the soil and stored in polyethylene bags at 4 °C, until field use.

#### 2.2.4. Mycorrhizal inoculum potential of the experimental field soil and of AM fungal inocula

The infectivity of the experimental field soil and of the AM fungal inocula was tested using mycorrhizal inoculum potential (MIP) test: *Lactuca sativa* seeds were sown in 50 mL sterile plastic tubes filled with 40 mL of each inocula (IMA1, AZ225C, IMA5, IMA6, EMix, Nmix) and of the experimental field soil obtained by mixing one soil subsample from each experimental field plot, removed to a depth of 30 cm using a soil corer (8 cm diameter), and air dried. Six replicate plastic tubes were used for each inoculum and the field soil. After emergence, *L. sativa* plants were thinned to 3. Plants were removed from tubes after two weeks' growth and root systems stained (see Experiment 1), mounted on microscope slides and examined under a Reichert-Jung (Vienna, Austria) Polyvar microscope. Root length and colonized root length were measured using a grid eyepiece. Number of infection units measured as hyphae with entry points, and number of entry points were assessed at magnifications of ×125–500 and verified at a magnification of ×1250.

#### 2.2.5. Field experimental setup

Plots (5 m × 3 m) were dug (10 cm depth) and harrowed (5 cm depth) in September 2004 and then inoculated with 10.5 kg plot$^{-1}$ of crude inoculum for each treatment. *T. alexandrinum* plots were sown with 5 g m$^{-2}$ of seeds. *T. alexandrinum* was harvested after eight month's growth and then one month later. The experiment was a completely randomized design with seven fungal treatments (IMA1, AZ225C, IMA5, IMA6, EMix, NMix and a control), two harvests and three replicates.

#### 2.2.6. Plant growth response and nutrient uptake

One month after emergence, a sample of *T. alexandrinum* plants ($n = 6$) for each replicate plot was carefully removed from the soil. Percentage of AM fungal root colonization was assessed, after staining, using the gridline intersect method (see Experiment 1). At both harvests, *T. alexandrinum* shoots from one square metre for each replicate plot were air dried. Dry weights of stems and leaves, shoot N and P concentration and content were determined (per square metre) as described above (Experiment 1). A root sample extracted from soil (100 g fresh weight) of the same plot was assessed for mycorrhizal colonization (see Experiment 1). At the second harvest, the number of stems regrown from each plant was also determined in each square metre. Seed production was determined after a further three week's growth.

### 2.3. Experiment 3: field evaluation of growth response and nutrient uptake of Z. mays as following crop

This experiment aimed at evaluating growth and nutritional responses of *Z. mays* L. cv. Eleonora, grown in rotation with *T. alexandrinum* in the same field plots of Experiment 2.

Plots were dug (10 cm depth) and harrowed (5 cm depth) in April 2006, and maize sown in May 2006 to obtain a plant density of 8.7 plants m$^{-2}$. At harvest, in September 2006, *Z. mays* biomass was assessed as shoot dry weight after ear removal. Measurements were performed on 3 plants for each plot, by drying at 58 °C for seven days. Percentage of AM fungal colonization was assessed (see Experiment 1). A sample of two rows for each replicate plot was used to evaluate the number of ears per plant. Maize grain weight was assessed on a subsample of two ears per plot, and production per square metre was calculated. Moreover, the dry weight of 1000 seeds (grain yield) was used as a parameter of maize quality. N and P concentration and content were determined on grains as described above (Experiment 1).

### 2.4. Statistics and data analyses

Data of all experiments were analyzed by one-way (fungal treatment as factor) analysis of variance (ANOVA). Data were ln- or arcsine-transformed when needed to fulfil the assumptions of the ANOVA, which was carried out according to the completely randomized design. Multiple comparisons within the ANOVAs were made with orthogonal contrasts. Analysis of covariance (ANCOVA) was used to separate the effects of the different treatments on AM fungal colonization percentage from those on physiological parameters, using the percentage of root colonization as covariate. Since ANCOVA showed no effect of root colonization on treatments, only ANOVA results were reported. Analyses were performed with the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Means and standard errors (S.E.) given in tables are for untransformed data.

Data of each experiment were evaluated in constrained ordination analyses (Redundancy Analysis, RDA) (van den Wollenberg, 1977) in order to investigate the influence of the different treatments in greenhouse and field (used as explanatory variables) on plant physiological variables (used as response variables) by the percentages of root colonization as covariable. RDA analyses were performed with Canoco for Windows v. 4.5 (ter Braak and Smilauer, 2002). Additionally, Monte Carlo permutation tests were conducted using 499 random permutations in order to determine the statistical significance of the relation between the whole set of explanatory variables and the plant physiological variables.

## 3. Results

### 3.1. Evaluation of plant physiological traits as affected by four exotic AMF inoculated on T. alexandrinum (Experiment 1)

#### 3.1.1. Mycorrhizal colonization

Three months after inoculation, the four *Glomus* isolates successfully established mycorrhizal symbiosis with *T. alexandrinum*, which

**Table 1**
Shoot, leaf, stem dry weights of *Trifolium alexandrinum* plants inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), and not inoculated (NM). Plants grown in microcosm for four months.

| | Shoot dry matter (g pot$^{-1}$) | Leaf dry matter (g pot$^{-1}$) | Stem dry matter (g pot$^{-1}$) |
|---|---|---|---|
| NM | 1.53 ± 0.13[a] | 0.99 ± 0.08 | 0.54 ± 0.05 |
| AZ225C | 3.53 ± 0.15 | 2.05 ± 0.08 | 1.48 ± 0.12 |
| IMA1 | 3.81 ± 0.16 | 2.17 ± 0.07 | 1.64 ± 0.09 |
| IMA5 | 3.27 ± 0.15 | 1.94 ± 0.11 | 1.33 ± 0.04 |
| IMA6 | 3.38 ± 0.34 | 2.00 ± 0.22 | 1.39 ± 0.16 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | |
| NM vs M | **<0.001** | **<0.001** | **<0.001** |
| *G. mosseae* vs *G. intraradices* | 0.086 | 0.218 | 0.079 |
| IMA1 vs AZ225C | 0.239 | 0.356 | 0.260 |
| IMA5 vs IMA6 | 0.706 | 0.759 | 0.729 |

NM, non-mycorrhizal; M, mycorrhizal.
[a] Values are means ± SE of five replicate pots for each treatment; in bold, statistically significant values ($P < 0.05$).

**Table 2**
Root response variables of *Trifolium alexandrinum* inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225Cand IMA1) and *Glomus intraradices* (IMA5 and IMA6), and not inoculated (NM). Plants grown in microcosms for four months.

| | Root dry matter (g pot$^{-1}$) | Root P concentration | Root P content (mg pot$^{-1}$) |
|---|---|---|---|
| NM | 0.58 ± 0.05[a] | 1.18 ± 0.05 | 0.67 ± 0.05 |
| AZ225C | 1.10 ± 0.09 | 1.63 ± 0.07 | 1.78 ± 0.10 |
| IMA1 | 1.07 ± 0.11 | 1.95 ± 0.13 | 2.04 ± 0.14 |
| IMA5 | 0.77 ± 0.05 | 2.06 ± 0.01 | 1.59 ± 0.11 |
| IMA6 | 1.08 ± 0.14 | 2.08 ± 0.17 | 2.19 ± 0.13 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | |
| NM vs M | **<0.001** | **<0.001** | **<0.001** |
| *G. mosseae* vs *G. intraradices* | 0.120 | **0.018** | 0.860 |
| IMA1 vs AZ225C | 0.790 | **0.027** | 0.093 |
| IMA5 vs IMA6 | 0.067 | 0.927 | **0.006** |

NM, nonmycorrhizal; M, mycorrhizal.
[a] Values are means ± SE of five replicate pots for each treatment; in bold, statistically significant values ($P \leq 0.05$).

showed also root nodules. Percentages of colonized root length ranged from 51.8 to 65.7% (in *G. mosseae* IMA1 and AZ225C inoculated plants, respectively), and from 76.1 to 92.7% (in *G. intraradices* IMA6 and IMA5 inoculated plants, respectively). No mycorrhizal colonization was observed in control plants. Interestingly, *G. mosseae* and *G. intraradices* inoculated clover showed significantly different mycorrhizal colonization levels both between ($P < 0.001$) and within species ($P = 0.002$, for *G. mosseae*; $P < 0.001$, for *G. intraradices*).

### 3.1.2. Plant growth

Host benefits, calculated for each fungal isolate as ((dry weight mycorrhizal plant − dry weight non-mycorrhizal plant)/dry weight non-mycorrhizal plant) × 100, were 131, 149, 114, 121% for *G. mosseae* AZ225C and IMA1, *G. intraradices* IMA5 and IMA6. Shoot and root biomass productions were significantly higher in mycorrhizal plants (Tables 1 and 2).

### 3.1.3. N and P uptake

Mycorrhizal *T. alexandrinum* plants showed significantly higher shoot N and P concentrations and contents compared with control plants (Table 3). Shoot N concentration and both P concentration and content were affected by AM fungal species ($P = 0.003$, $P < 0.001$, and $P < 0.001$, respectively) (Table 3). At the intraspecific level, *G. intraradices* IMA5 showed the highest values for all plant variables, except for shoot N content, while *G. mosseae* isolates did not differ (Table 3).

P root concentrations and contents were significantly different in mycorrhizal plants, compared with controls (Table 2). Significant differences were observed between the two *Glomus* species ($P = 0.018$), and, at intraspecific level, within *G. mosseae* ($P = 0.027$) for root P concentration. Moreover, root P content significantly differed between the two *G. intraradices* isolates ($P = 0.006$).

### 3.1.4. Main patterns of plant physiological traits as affected by AMF

The influence of fungal treatments on growth and nutrient uptake, measured to describe host benefit, was investigated using a multivariate statistical approach. The RDA for *T. alexandrinum* showed that the fungal treatments explained 83.5% (I and II axes) of the whole variance and that their effect on plant physiological variables was significant ($P = 0.002$). In detail, Monte Carlo permutation test showed that the control was significantly different from the other fungal treatments ($P = 0.002$) and that IMA5 was significantly different from the other AM fungal isolates ($P = 0.002$). Moreover, AZ225C isolate was not significantly different from IMA1 and IMA6 ($P = 0.054$). The biplot diagram of the RDA (Fig. 1a) shows these results: the centroid of the control is distant from the other AM fungal treatments, and the centroid representing IMA5 is distant from the other AM fungal isolates. The biplot also shows that shoot N content represents the most discriminating variable between AM fungal treatments and the control (Fig. 1a). In addition, shoot N concentration, higher in the plants inoculated with IMA5, can be used to distinguish this isolate from the others (Fig. 1a). The diagram points out also correlations between shoot, leaf and stem dry matter, as expected, and between root P concentration and shoot P content (Fig. 1a). Consistent results were obtained when colonized root percentages were utilised in RDA as covariable, although, in this case, 59.1% (I and II axes) of the whole variance was explained by fungal treatments.

## 3.2. Field evaluation of growth responses and nutrient uptake of *T. alexandrinum* inoculated with exotic and native AM fungal inocula (Experiment 2)

### 3.2.1. Mycorrhizal inoculum potential (MIP) of the experimental field soil and AM fungal inocula

MIP of the experimental field soil showed values of 0.23 ± 0.03 and 0.24 ± 0.03 infection units and entry points cm$^{-1}$ root length, respectively. MIP values of mixed and single inocula ranged from 1.10 ± 0.34 to 2.89 ± 0.54 infection units cm$^{-1}$ root length in IMA5 and IMA1, respectively, and from 1.30 ± 0.45 to 4.48 ± 0.70 entry points cm$^{-1}$ root length in NMix and IMA1, respectively.

### 3.2.2. Mycorrhizal colonization

One month after emergence, percentage of mycorrhizal root length of control plants was 5.0 ± 0.9%, significantly differing from

**Table 3**
Shoot N and P concentration and content of *Trifolium alexandrinum* plants inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), and not inoculated (NM). Plants grown in microcosm for four months.

| | Shoot N concentration (%) | Shoot P concentration (‰) | Shoot N content (mg pot$^{-1}$) | Shoot P content (mg pot$^{-1}$) |
|---|---|---|---|---|
| NM | 2.07 ± 0.03[a] | 0.83 ± 0.04 | 31.72 ± 2.87 | 1.27 ± 0.11 |
| AZ225C | 2.06 ± 0.03 | 1.31 ± 0.03 | 72.60 ± 3.13 | 4.60 ± 0.14 |
| IMA1 | 2.13 ± 0.05 | 1.32 ± 0.07 | 81.22 ± 3.51 | 4.98 ± 0.17 |
| IMA5 | 2.35 ± 0.01 | 2.18 ± 0.09 | 76.99 ± 3.62 | 7.09 ± 0.20 |
| IMA6 | 2.13 ± 0.02 | 1.31 ± 0.05 | 72.28 ± 7.35 | 4.43 ± 0.50 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | | |
| NM vs M | **0.025** | **<0.001** | **<0.001** | **<0.001** |
| *G. mosseae* vs *G intraradices* | **0.003** | **<0.001** | 0.586 | **<0.001** |
| IMA1 vs AZ225C | 0.154 | 0.902 | 0.106 | 0.182 |
| IMA5 vs IMA6 | **0.004** | **<0.001** | 0.478 | **<0.001** |

NM, non-mycorrhizal; M, mycorrhizal.
[a] Values are means ± SE of five replicate pots for each treatment; in bold, statistically significant values ($P < 0.05$).

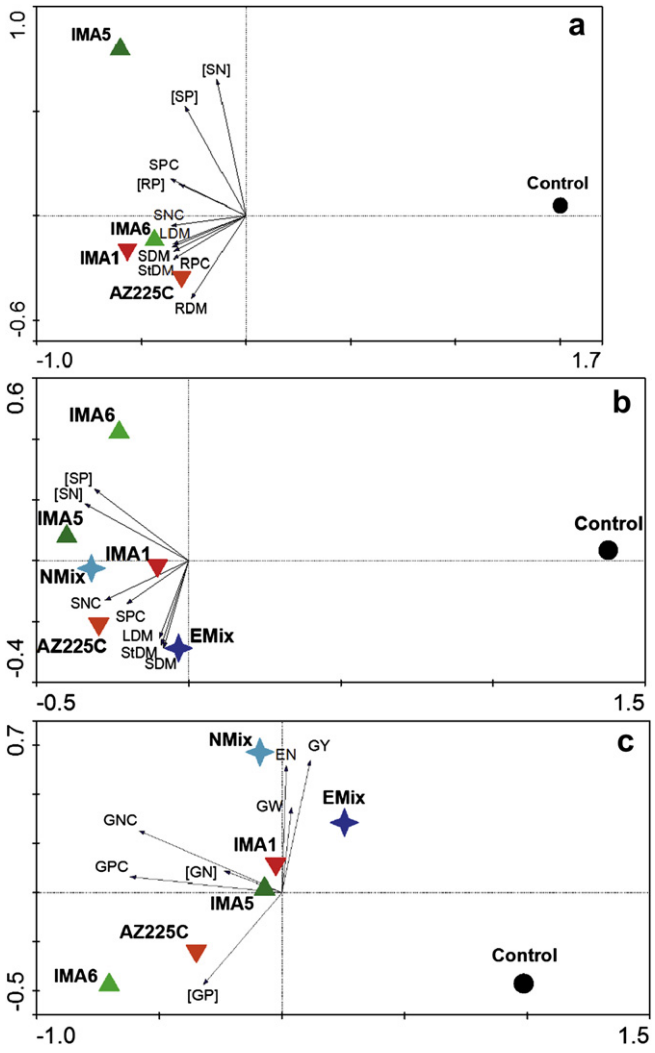


**Fig. 1.** Redundancy Analysis (RDA) biplot based on plant physiological variables and fungal treatments (focusing on inter-sample distances) of three datasets: (a) greenhouse evaluation of growth response and nutrient uptake of *Trifolium alexandrinum* inoculated with *Glomus intraradices* IMA5 and IMA6, *Glomus mosseae* AZ225C and IMA1 and of control; (b) field evaluation of growth response and nutrient uptake of *T. alexandrinum* inoculated with single exotic arbuscular mycorrhizal (AM) fungal isolate (AZ225C, IMA1, IMA5, IMA6), exotic mixed inoculum (EMix), native inoculum (NMix) and of control; (c) field evaluation of growth response and nutrient uptake of *Zea mays* as following crop inoculated with single exotic AM fungal isolate (AZ225C, IMA1, IMA5, IMA6), mixed inoculum (EMix), native inoculum (NMix) and of control. Treatments (explanatory variables) are represented by triangles (IMA5 and IMA6), down-triangles (AZ225C and IMA1), stars (EMix and NMix) and circles (Control). Plant physiological variables are represented by arrows: SDM (shoot dry matter), LDM (leaf dry matter), StDM (stem dry matter), RDM (root dry matter), [SN] (shoot nitrogen concentration), SNC (shoot N content), [SP] (shoot P concentration), SPC (shoot P content), [RP] (root P concentration) and RPC (root P content) (a); SDM, LDM, StDM, [SN], SNC, [SP] and SPC (b); GY (grain yield), GW (grain weight), NE (number of ears), [GN] (grain N concentration), GNC (grain N content), [GP] (grain P concentration) and GPC (grain P content). The 1st and 2nd axes accounted for 83.5% (a), 70.3% (b), 34.7% (c) of the variability explained by all canonical axes and were significant ($P = 0.002$) (a; b; c).

that of mycorrhizal treatments ($P < 0.001$) which ranged from $30.8 \pm 6.6\%$ to $53.8 \pm 7.7\%$ (NMix and IMA5, respectively). At first and second harvest, mycorrhizal colonization was significantly affected by AM fungal inoculation ($P < 0.001$ and $P < 0.001$, respectively) (Table 4). Interestingly, at the second harvest differences were observed between EMix and NMix treatment ($P = 0.007$) (Table 4). Root nodules were observed in all plant samples.

### 3.2.3. Plant growth

One month after emergence, no differences were observed in shoot, leaf and stem biomass of inoculated and control *T. alexandrinum* plants and among inoculated treatments (data not shown).

At the first harvest, host benefits were 99, 42, 73, 40, 52, 16% for EMix, NMix, *G. mosseae* AZ225C and IMA1, *G. intraradices* IMA5 and IMA6. Shoot biomass production was significantly affected by AM fungal inoculation ($P = 0.002$): mixed inocula differed from single inocula ($P = 0.043$) and from each other ($P = 0.009$) (Table 5). Interestingly, differences in leaf dry matter were observed at specific level, between *G. mosseae* and *G. intraradices* ($P = 0.001$), and at intraspecific level, both within *G. mosseae* and *G. intraradices* isolates ($P = 0.040$ and $P = 0.032$, respectively), while stem biomass differences were found in mixed vs single inocula treatments ($P = 0.043$) (Table 5).

At the second harvest, control and mycorrhizal treatments showed significant differences in shoot and leaf biomass productions ($P = 0.040$ and $P = 0.022$, respectively) (Table 5). The numbers of stems produced during regrowth were significantly higher in inoculated clover than in control plants, and in NMix than in EMix treated plants ($P = 0.012$) (Table 6).

Seed production was significantly affected by mycorrhizal inoculation (Table 6). Interestingly, differences at intraspecific level were observed between *G. mosseae* isolates ($P = 0.008$), as plants treated with *G. mosseae* AZ225C produced higher seed dry weight (Table 6).

### 3.2.4. N and P uptake

Inoculated plants showed significantly higher shoot N and P concentrations and contents, compared with control plants, both at first and second harvest (Table 7). However, mycorrhizal treatments did not significantly differ among each other, except, at the second harvest, where EMix produced higher shoot P content ($P = 0.016$) (Table 7).

### 3.2.5. Main patterns of plant physiological traits as affected by AMF

RDA showed that fungal treatments explained 70.3% (I and II axes) of the whole variance and that their effects on plant physiological variables were significant ($P = 0.002$). In detail, Monte Carlo permutation test showed that control and IMA6 were significantly different from the other treatments ($P = 0.002$), as well as EMix from the remaining fungal factors ($P = 0.044$). The biplot diagram of the RDA (Fig. 1b) shows these results: the centroid of the control is distant from AM fungal treatments; the centroids representing IMA6 and EMix are distant from each other and from the other AM fungal treatments. The biplot also shows that shoot N concentration was the most discriminating variable between AM fungal treatments and the control (Fig. 1b). In addition, shoot dry matter discriminates EMix and IMA6 from the other treatments (Fig. 1b). Leaf and stem dry matter are highly correlated with shoot dry matter (Fig. 1b). Consistent results were obtained when colonized root percentages were utilised in RDA as covariable, although, in this case, fungal treatments explained 55.2% (I and II axes) of the whole variance.

## 3.3. Field evaluation of growth response and nutrient uptake of Z. mays as following crop (Experiment 3)

### 3.3.1. Mycorrhizal colonization, plant growth and N and P uptake

At harvest, mycorrhizal colonization was not affected by inoculation, although contrasts showed significant differences between EMix and NMix treatments ($P = 0.028$) (Table 4). Maize growth and nutrient uptake variables were not affected by mycorrhizal treatments (data not shown). Though, NMix treatment consistently showed the best performances, which were: grain yield, $451 \pm 39$ g m$^{-2}$; number of

**Table 4**
Mycorrhizal colonization of *Trifolium alexandrinum* and *Zea mays* plants inoculated with a mixture of exotic (EMix) and native (NMix) arbuscular mycorrhizal (AM) fungi and with four isolates of the AM fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), and control (C). Field harvests of *T. alexandrinum* and *Z. mays* plants after eight (first harvest) and nine months (second harvest), and after six months (at the harvest), respectively.

| | *T. alexandrinum* mycorrhizal colonization 1st harvest (%) | *T. alexandrinum* mycorrhizal colonization 2nd harvest (%) | *Z. mays* mycorrhizal colonization at the harvest (%) |
|---|---|---|---|
| C | 12.8 ± 1.5[a] | 13.2 ± 0.8 | 39.7 ± 4.3 |
| EMix | 27.0 ± 5.7 | 30.8 ± 2.6 | 56.2 ± 2.1 |
| NMix | 39.8 ± 3.4 | 49.5 ± 4.6 | 35.9 ± 6.6 |
| AZ225C | 38.5 ± 6.6 | 29.8 ± 2.8 | 42.7 ± 7.6 |
| IMA1 | 31.2 ± 3.9 | 38.0 ± 4.4 | 47.6 ± 7.6 |
| IMA5 | 41.6 ± 5.4 | 36.7 ± 7.0 | 32.7 ± 5.1 |
| IMA6 | 40.5 ± 5.3 | 34.3 ± 4.2 | 44.5 ± 5.5 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | |
| C vs M | **<0.001** | **<0.001** | 0.604 |
| Mix vs Single | 0.296 | 0.154 | 0.424 |
| EMix vs NMix | 0.080 | **0.007** | **0.028** |
| *G. mosseae* vs *G. intraradices* | 0.220 | 0.704 | 0.277 |
| IMA1 vs AZ225C | 0.303 | 0.188 | 0.560 |
| IMA5 vs IMA6 | 0.874 | 0.696 | 0.165 |

M, mycorrhizal; Mix, mixed inocula; Single, single inocula.
[a] Values are means ± SE of five replicate pots for each treatment; in bold, statistically significant values ($P < 0.05$).

**Table 5**
Shoot, leaf, stem dry weights of *Trifolium alexandrinum* plants inoculated with a mixture of exotic (EMix) and native (NMix) arbuscular mycorrhizal (AM) fungi and with four isolates of the AM fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), and control (C). Plants harvested after eight and nine months in the field (first and second harvest).

| | Shoot dry matter (g $m^{-2}$) | Leaf dry matter (g $m^{-2}$) | Stem dry matter (g $m^{-2}$) |
|---|---|---|---|
| 1st harvest | | | |
| C | 495.7 ± 76.0[a] | 255.3 ± 25.3 | 240.4 ± 72.9 |
| EMix | 988.4 ± 58.7 | 420.7 ± 37.5 | 567.6 ± 23.8 |
| NMix | 706.1 ± 63.5 | 340.1 ± 62.4 | 366.0 ± 64.9 |
| AZ225C | 857.8 ± 61.3 | 458.1 ± 17.9 | 399.7 ± 48.9 |
| IMA1 | 696.0 ± 76.8 | 380.8 ± 2.8 | 315.2 ± 48.9 |
| IMA5 | 752.2 ± 84.7 | 368.2 ± 11.4 | 384.0 ± 94.7 |
| IMA6 | 576.5 ± 9.9 | 316.6 ± 2.6 | 259.9 ± 11.6 |
| 2nd harvest | | | |
| C | 156.0 ± 19.1 | 80.8 ± 10.0 | 75.2 ± 9.6 |
| EMix | 258.4 ± 6.5 | 142.3 ± 6.9 | 116.0 ± 3.4 |
| NMix | 311.9 ± 97.2 | 152.8 ± 42.9 | 159.1 ± 54.3 |
| AZ225C | 301.1 ± 21.9 | 177.0 ± 11.5 | 124.2 ± 10.4 |
| IMA1 | 263.6 ± 41.5 | 138.2 ± 22.3 | 125.4 ± 19.2 |
| IMA5 | 239.3 ± 33.4 | 125.7 ± 15.5 | 113.6 ± 18.0 |
| IMA6 | 172.4 ± 52.5 | 96.4 ± 29.1 | 76.0 ± 23.4 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | |
| 1st harvest | | | |
| C vs M | **0.002** | **0.043** | 0.222 |
| Mix vs Single | **0.043** | 0.871 | **0.043** |
| EMix vs NMix | **0.009** | 0.317 | 0.102 |
| *G. mosseae* vs *G. intraradices* | 0.109 | **0.001** | 0.575 |
| IMA1 vs AZ225C | 0.103 | **0.040** | 0.365 |
| IMA5 vs IMA6 | 0.079 | **0.032** | 0.399 |
| 2nd harvest | | | |
| C vs M | **0.040** | **0.022** | 0.078 |
| Mix vs Single | 0.332 | 0.473 | 0.225 |
| EMix vs NMix | 0.710 | 0.994 | 0.465 |
| *G. mosseae* vs *G. intraradices* | 0.069 | **0.048** | 0.107 |
| IMA1 vs AZ225C | 0.574 | 0.309 | 0.979 |
| IMA5 vs IMA6 | 0.152 | 0.200 | 0.114 |

M, mycorrhizal; Mix, mixed inocula; Single, single inocula.
[a] Values are means ± SE of three replicate plots for each treatment; in bold, statistically significant values ($P \leq 0.05$).

ears, 6.8 ± 0.4 $m^{-2}$, grain weight, 170 ± 6 g thousand $seeds^{-1}$; N content, 4.5 ± 0.5 and P content 1.5 ± 0.1 g $m^{-2}$.

### 3.3.2. Main patterns of plant physiological traits as affected by AMF

RDA showed that the fungal treatments explained 34.7% (I and II axes) of the whole variance and that their effect on plant physiological variables was significant ($P = 0.002$). In detail, Monte Carlo permutation test showed that the control was significantly different from the fungal isolates treatments ($P = 0.006$). The biplot diagram of the RDA shows that the centroids representing AM fungal treatments are distant from the control (Fig. 1c). Grain P content is the most discriminating variable on the first axes (Fig. 1c). Consistent results were obtained when colonized root percentages were utilised in RDA as covariable, although, in this case, fungal treatments explained 39.7% (I and II axes) of the whole variance.

## 4. Discussion

In this work, we reported beneficial effects of field inoculation of exotic and native AM fungal isolates in a low input agroecosystem.

**Table 6**
Regrowth ability and seed dry weight of *Trifolium alexandrinum* plants inoculated with a mixture of exotic (EMix) and native (NMix) arbuscular myeorrhizal (AM) fungi and with four isolates of the AM fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA), and control (C). Plants harvested after eight and nine months in the field (first and second harvest).

| | Regrowth ability (stems $plant^{-1}$) | Seed dry weight (g $m^{-2}$) |
|---|---|---|
| C | 2.06 ± 0.08[a] | 64.07 ± 4.57 |
| EMix | 2.86 ± 0.35 | 116.03 ± 30.68 |
| NMix | 4.30 ± 0.47 | 129.00 ± 10.66 |
| AZ225C | 3.66 ± 0.05 | 129.63 ± 6.10 |
| IMA1 | 3.20 ± 0.56 | 90.87 ± 2.12 |
| IMA5 | 2.91 ± 0.09 | 118.97 ± 15.70 |
| IMA6 | 3.88 ± 0.44 | 123.93 ± 9.53 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | |
| C vs M | **0.002** | **0.002** |
| Mix vs Single | 0.579 | 0.827 |
| EMix vs NMix | **0.012** | 0.588 |
| *G mosseae* vs *G. intraradices* | 0.910 | 0.258 |
| IMA1 vs AZ225C | 0.375 | **0.008** |
| IMA5 vs IMA6 | 0.071 | 0.749 |

M, mycorrhizal; Mix, mixed inocula; Single, single inocula.
[a] Values are means ± SE of three replicate plots for each treatment; in bold, statistically significant values ($P \leq 0.05$).

**Table 7**
Shoot N and P concentration and content of *Trifolium alexandrinum* plants inoculated with a mixture of exotic (EMix) and native (NMix) arbuscular mycorrhizal (AM) fungi and with four isolates of the AM fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), and control (C). Plants harvested after eight and nine months in the field (first and second harvest).

| | Shoot N concentration (%) | Shoot P concentration (‰) | Shoot N content (g $m^{-2}$) | Shoot P content (g $m^{-2}$) |
|---|---|---|---|---|
| 1st harvest | | | | |
| C | 0.56 ± 0.06[a] | 1.29 ± 0.13 | 2.84 ± 0.76 | 0.63 ± 0.08 |
| EMix | 1.84 ± 0.25 | 2.30 ± 0.10 | 18.32 ± 3.10 | 2.28 ± 0.21 |
| NMix | 2.31 ± 0.27 | 3.13 ± 0.47 | 15.87 ± 2.09 | 2.17 ± 0.27 |
| AZ225C | 2.31 ± 0.46 | 2.63 ± 0.12 | 19.57 ± 1.55 | 2.25 ± 0.08 |
| IMA1 | 2.02 ± 0.31 | 2.80 ± 0.46 | 13.59 ± 0.94 | 1.88 ± 0.14 |
| IMA5 | 2.60 ± 0.35 | 3.27 ± 0.58 | 19.31 ± 2.92 | 2.38 ± 0.28 |
| IMA6 | 2.74 ± 0.20 | 3.23 ± 0.37 | 15.78 ± 1.05 | 1.86 ± 0.21 |
| 2nd harvest | | | | |
| C | 0.76 ± 0.06 | 1.43 ± 0.15 | 1.17 ± 0.12 | 0.23 ± 0.05 |
| EMix | 2.04 ± 0.25 | 2.47 ± 0.07 | 5.25 ± 0.52 | 0.64 ± 0.01 |
| NMix | 2.51 ± 0.41 | 3.33 ± 0.47 | 7.06 ± 0.90 | 0.95 ± 0.14 |
| AZ225C | 2.51 ± 0.27 | 2.80 ± 0.15 | 7.61 ± 1.14 | 0.85 ± 0.10 |
| IMA1 | 2.22 ± 0.31 | 3.03 ± 0.43 | 5.64 ± 0.58 | 0.77 ± 0.05 |
| IMA5 | 2.80 ± 0.35 | 3.47 ± 0.58 | 6.51 ± 0.66 | 0.79 ± 0.04 |
| IMA6 | 2.94 ± 0.20 | 3.37 ± 0.34 | 4.86 ± 1.15 | 0.55 ± 0.11 |
| Treatments compared (*P*-values of linear orthogonal contrasts) | | | | |
| 1st harvest | | | | |
| C vs M | **<0.001** | **0.001** | **<0.001** | **<0.001** |
| Mix vs Single | 0.184 | 0.414 | 0.985 | 0.444 |
| EMix vs NMix | 0.266 | 0.130 | 0.396 | 0.705 |
| *G. mosseae* vs *G. intraradices* | 0.098 | 0.168 | 0.636 | 0.765 |
| IMA1 vs AZ225C | 0.475 | 0.753 | 0.051 | 0.210 |
| IMA5 vs IMA6 | 0.725 | 0.950 | 0.228 | 0.083 |
| 2nd harvest | | | | |
| C vs M | **<0.001** | **0.001** | **<0.001** | **<0.001** |
| Mix vs Single | 0.184 | 0.404 | 0.994 | 0.442 |
| EMix vs NMix | 0.266 | 0.109 | 0.132 | **0.016** |
| *G. mosseae* vs *G. intraradices* | 0.098 | 0.184 | 0.257 | 0.112 |
| IMA1 vs AZ225C | 0.476 | 0.652 | 0.103 | 0.493 |
| IMA5 vs IMA6 | 0.725 | 0.846 | 0.167 | **0.050** |

M, mycorrhizal; Mix, mixed inocula; Single, single inocula.
[a] Values are means ± SE of three replicate plots for each treatment; in bold, statistically significant values ($P \leq 0.05$).

Multivariate analyses showed that (i) all AM fungal inocula increased plant productivity and nutrient content, compared with controls, (ii) the native inoculum was as effective as, or more than, exotic AM fungal isolates (iii) beneficial effects of all AM fungal inocula were persistent until the second year after inoculation.

### 4.1. Evaluation of plant physiological traits as affected by four exotic AMF inoculated on T. alexandrinum

In this work *T. alexandrinum* showed a high degree of mycotrophy, since all fungal treatments improved shoot biomass, N and P contents and concentrations, compared with control plants. Here, all *T. alexandrinum* plants showed root nodules, whose effect on the plant's nitrogen budget, via biological nitrogen fixation (BNF), can be influenced by AM fungal colonization (Mortimer et al., 2009; Kaschuk et al., 2009, 2010). On the other hand, as nodules are large sinks for P, the observed differences between inoculated and uninoculated plants in root P content may also be due to synergistic AM fungi–rhizobia interactions (Azcón-Aguilar and Barea, 1981; Al-Niemi et al., 1998; Almeida et al., 2000; Jia et al., 2004). We observed differences in plant responses between and within the two AM fungal species studied. RDA analysis based on plant physiological variables showed that, while *G. mosseae* isolates did not differ, the two *G. intraradices* isolates were significantly different. A large functional intraspecific diversity was previously shown, confirming that mycorrhizal benefits vary depending on the genetic diversity of AM fungal isolates (Jakobsen et al., 1992a; Smith et al., 2000; Streitwolf-Engel et al., 2001; van der Heijden and Sanders, 2002; Munkvold et al., 2004; Koch et al., 2006). However, as suggested by Tchabi et al. (2010), host growth promotion may vary also depending on factors related to inoculum preparation.

*G. intraradices* isolates were more effective in root colonization than *G. mosseae* isolates, consistently with previous data on *Medicago sativa* (Vazquez et al., 2001; Avio et al., 2006; Bedini et al., 2009). Moreover, *G. intraradices* IMA5 showed higher colonization ability on *T. alexandrinum* than on *M. sativa* (Avio et al., 2006).

Here, we found inter- and intraspecific functional differences among the AM fungal isolates inoculated on *T. alexandrinum* in relation to plant P content and N and P concentrations. Interestingly, *G. intraradices* IMA5 was the most effective isolate as to P uptake, since P concentrations of inoculated plants increased by 66%, compared with the other isolates. So far, no information was available on the effects of different AM fungal isolates on *T. alexandrinum* growth response and nutrient uptake, whereas large inter- and intraspecific functional diversity was previously observed in a different legume crop, *M. sativa* (Avio et al., 2006; Vazquez et al., 2001). By contrast, Zhao et al. (1997) did not detect any interspecific difference between *G. mosseae* and *G. intraradices* in symbiosis with other legume crops, such as *Glycine max* and *Astragalus sinicum*, confirming that mycorrhizal effectiveness may also depend on host plants (Klironomos, 2000, 2003; Helgason et al., 2002).

### 4.2. Field responses of T. alexandrinum to inoculation of exotic and native AMF

In the field, AM fungal inoculation increased *T. alexandrinum* productivity and quality, compared with controls. Root colonization

of inoculated plants increased by 177% compared with controls, showing that the low level of mycorrhizal potential of the experimental soil could be successfully increased by means of inoculation. Even if root colonization percentage does not always represent a good predictor of plant productivity, a recent meta-analysis showed that mycorrhizal colonization increases might lead to higher plant benefits (Lekberg and Koide, 2005). At the end of the cultivation cycle, shoot dry biomass of *T. alexandrinum* was about 6.5 and 10 t $ha^{-1}$ in control and inoculated plots, respectively, yields that are comparable with those obtained in non irrigated Italian agricultural systems (Bonciarelli, 1998; Martiniello, 1998, 1999). Interestingly, seed dry weight of inoculated plots was much larger than that of control plots, 1.18 t $ha^{-1}$ compared with 0.64 t $ha^{-1}$.

#### 4.2.1. Field effectiveness of four exotic Glomus isolates

Our data show that, as previously observed in the greenhouse experiment, the two *G. intraradices* isolates were functionally different. In detail, as to root colonization ability, univariate analysis showed no differences among AM fungal isolates in the field. These data cannot be compared with those obtained in greenhouse, since the competition between introduced and native AM fungal isolates may lead to unpredictable results. Actually, a dominant effect of the more competitive among three coinoculated AMF was observed in a greenhouse experiment (Jansa et al., 2008).

No differences were observed in the shoot biomass of *T. alexandrinum* inoculated with the four AM fungal isolates, whereas interspecific and intraspecific variability in the production of leaves was detected. Interspecific differences in shoot biomass were previously observed between *Glomus fasciculatum* and *Gigaspora margarita*, and between *G. mosseae* and *G. etunicatum*, in association with *Trifolium repens* (Powell, 1977; Rangeley et al., 1982). Abbott et al. (1983) found differences in the productivity of *Trifolium subterraneum* inoculated with *G. fasciculatum* and *Glomus monosporum*, only at the first harvest, whereas, in our experiment, interspecific variability was observed at both harvests. In addition, in our experiment no differences in nutrient uptake were detected, except for shoot P content between *G. intraradices* IMA5 and IMA6 at the second harvest. This lack of nutrient uptake variability between species is consistent with the results of Powell (1977) and Rangeley et al. (1982), but in contrast with data obtained in our previous greenhouse experiment. The predictive power of such an experiment, for field outcomes, was evaluated by Mantel test, using growth and nutrient uptake data of each AM fungal isolate obtained in greenhouse and field experiments. The test showed that field outcomes were not correlated with greenhouse *T. alexandrinum* growth responses (Mantel test: $r = -0.06$; $P = 0.56$). Pringle and Bever (2008) observed a strong predictive power of laboratory data obtained with *Allium vineale* inoculated with several AM fungal species, but they did not detect any relationship between field and laboratory biomasses for *Anthoxanthum odoratum* and *Rumex acetosella*.

#### 4.2.2. Field effectiveness of single and mixed AM fungal inocula

This is the first study showing in the field that native AM fungal inocula are as effective as highly efficient single exotic ones. Multivariate analysis showed that the native inoculum performed better than both the exotic mixed inoculum and *G. intraradices* IMA6, and similarly to the other exotic single isolates. In detail, mixed inocula (both native and exotic) produced higher shoot and stem biomass increases than single ones, at the first harvest. The influence of AM fungal species richness on plant productivity was studied in several microcosm experiments, while only few trials were carried out to evaluate its effects on crop performances in the field. Most microcosm studies reported that diversity and productivity of natural plant communities were positively correlated with AM fungal species richness (van der Heijden et al., 1998; Smith et al., 2000; Hart and Klironomos, 2002; Vogelsang et al., 2006; Gustafson and Casper, 2006). Though, some works reported that single AM fungal isolates gave similar or greater effect than mixed inocula (Daft and Hogarth, 1983; Pearson et al., 1994; Lekberg et al., 2007) and that native AM fungal isolates produced equal or superior beneficial effects than exotic isolates (Tchabi et al., 2010). In field studies, single exotic inocula were more efficient than mixed native ones (Powell, 1977, 1979; Sreeramulu and Bagyaraj, 1986). On the other hand, Requena et al. (2001) and Caravaca et al. (2005), found that, in natural ecosystems, field inoculation with native inocula was more effective than that with single exotic inocula. Such contrasting results may be explained by a dominant effect of one or more isolates of the complex AM fungal community determining the final plant performance (Jansa et al., 2008, 2009). Some of the differences observed in mycorrhizal responses of *T. alexandrinum* reported here may be explained by a synergistic effect of the mixed inocula (Koide, 2000; Smith et al., 2000), due to the complementarities of biological traits among AM fungal isolates, such as the extent and structure of mycorrhizal networks (Jakobsen et al., 1992a,b; Jakobsen et al., 2001; Avio et al., 2006), and by the differential induction of plant ammonium and P transporter genes in different AM fungal strains (Gamper et al., 2009).

### 4.3. Field responses of Z. mays as following crop

*Z. mays* grown in EMix plots showed significantly higher AM fungal colonization than *T. alexandrinum,* suggesting a differential compatibility between native and exotic AM fungal isolates and host plants (Klironomos, 2003; Helgason et al., 2007).

Our results show that mycorrhizal inocula were still effective after two years, suggesting their ability to persist in the soil for the period of the *T. alexandrinum*–*Z. mays* crop rotation. In *Z. mays* no differences were observed among inoculation treatments, and the native inoculum was as effective as the exotic AM fungal isolates. The persistence of introduced AM fungal inocula in soil is to be considered a key prerequisite for the success of inoculation practices. Although no long-term studies have been performed in agricultural soils, our results are consistent with those obtained in natural soils by Caravaca et al. (2005).

## 5. Conclusions

This study shows that the use of AM fungal inocula may be very effective in improving crop productivity and quality in low input agricultural systems and that their effects are persistent at least until two years after inoculation. Differences in isolate performances indicate that the choice of the best AM fungal inoculum for field utilisation is pivotal for the success of inoculation practices. Actually, multivariate analyses showed that native AM fungal inoculum is at least as effective as exotic isolates, which had been selected for their high efficiency. Therefore, the use of native AMF, produced on farm with mycotrophic plants species, might represent a convenient alternative to commercial AM fungal inocula, and offer economically and ecologically important advantages in sustainable or organic cropping systems.

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