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Field Functional Diversity of Arbuscular Mycorrhizal Fungi in a Crop Rotation of *Trifolium alexandrinum* and *Zea mays*

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Soil microbes play a major role in the functioning of agroecosystems. Arbuscular mycorrhizal fungi (AMF) are beneficial microbes fundamental in soil fertility and plant nutrition, enhancing plant P and N uptake by means of their extraradical mycelium (ERM) spreading from mycorrhizal roots into the surrounding soil (Smith and Read, 2008). Many studies have been carried out with plants grown in sterile soil with or without AMF inoculation (Avio *et al.*, 2006), while little is known about mycorrhizal symbiosis in nonsterile soils, which can contain diverse microorganisms differently influencing plant growth. Furthermore, the impact of agricultural practices on mycorrhizal colonization and host plant response in the field is not yet clearly understood. Greenhouse and field studies were performed in order to assess inter- and intraspecific functional diversity of geographically different isolates of the AMF species *Glomus intraradices* and *Glomus mosseae*, and of indigenous isolates inoculated on *Trifolium alexandrinum* and *Zea mays*, in a 2-year crop rotation.

Methodology

Plant and fungal material. The AMF used were: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolate IMA1 from UK and isolate AZ225C from USA, *Glomus intraradices* Schenck & Smith, isolate IMA5 from Italy and isolate IMA6 from France, inoculated singly (exp. 1, in greehouse and exp. 2, in the field) or as a mixture (Mix) (exp. 2), and a population of indigenous AMF (Indy) (exp. 2). The indigenous population has been morphologically and molecularly characterized by Pellegrino *et al.* (2007). The plant species used were *Trifolium alexandrinum* L. cv. Tigri (exp.1 and exp.2) and *Zea mays* L. cv. Eleonora (exp. 2). Greehouse experiment. Seeds of *T. alexandrinum* were sown into pots containing steam-sterilized soil and Terragreen. Pots were inoculated either with 90 ml of crude inoculum of one of the four isolates or with 90 ml of a sterilized mixture of them (control). All the pots received a filtrate to ensure a common microflora. The experiment was a completely randomized design with 5 inoculum treatments (fungal isolates and control), and 5 replicates. Three months after emergence, plant shoots were harvested, and *T. alexandrinum* dry weights were determined. Percentage of AMF colonization and total root length were assessed (Giovannetti and Mosse, 1980). N and P concentrations were assessed using Kjeldahl method and using the photometric method, respectively. Field experiment. In order to prepare the large quantity of inoculum to be used in the field experiment, AMF were reproduced in sterile soil and Terragreen, using *Z. mays* as host plant. The experimental field was prepared by digging and harrowing the soil, which was then inoculated with 0.7 Kg m^{-2} of crude inoculum or with a sterilized inoculum mixture (control). The experimental design was a randomized block with seven inoculum treatments, three replicates, three harvests for *T. alexandrinum* and one harvest for *Z. mays* (one year after AM fungal inoculation). Experimental field soil and inocula were tested for mycorrhizal potential (MIP) (Pellegrino *et al.*, 2007) and spore density. Short-term effect of AMF inoculum. At each harvest, dry weight, percentage of AMF colonization, number of stems, seed weight, and N and P concentrations were assessed. Long-term effect of AMF inoculum. *Z. mays* dry shoot matter, percentage of AMF colonization, number of ears $plant^{-1}$, grain dry weights, weight of 1000 seeds, grain N and P concentrations were assessed. Data of exp. 1 and exp. 2

were compared using one-way and two-way ANOVA, respectively. Data were trasformed when needed and multiple comparisons were done with Tukey's B test.

Results

Greehouse experiment. The four *Glomus* isolates successfully established mycorrhizal symbioses with *T. alexandrinum*. Host benefits, calculated as dry weight increases, were 131, 149, 114, 121% for *G. mosseae* AZ225C and IMA1, *G. intraradices* IMA5 and IMA6, respectively. Plants colonized by *G. mosseae* showed higher shoot dry weights than those colonized by *G. intraradices*, while plants inoculated with *G.intraradices* showed a larger stem biomass. Root biomass and length were significantly affected by mycorrhizal symbiosis and differences in root length were observed at inter- and intraspecific level. All mycorrhizal *T. alexandrinum* plants showed significantly higher N and P shoot concentrations compared with nonmycorrhizal controls. Differences in shoot N and P concentrations were observed at the interspecific (*G. intraradices* > *G. mosseae*) and the intraspecific level (IMA5 > IMA6). N and P shoot contents of mycorrhizal plants were significantly higher than those of controls. Host benefits calculated as N content increases, were 129, 156, 143, 128%, and calculated as P content increases were 262, 292, 458, 249%, for AZ225C, IMA1, IMA5 and IMA6, respectively. Moreover, differences in P content between the two species (*G. intraradices* > *G. mosseae*) and within *G. intraradices* species (IMA5 > IMA6) were observed.

Field experiment. Short term effect of AMF inoculation. The different AMF isolates used as inocula showed a significantly higher infectivity compared to the natural experimental soil. After one month's growth, mycorrhizal colonization was significantly higher in inoculated plants (43.5%) than in controls (5.0%), but it did not affect ecophysiological parameters. *T. alexandrinum* shoot biomass were significantly affected by mycorrhizal symbiosis at the first and second harvests. Host benefits, calculated as mean dry weights of the two harvests, were 78, 47, 52, 15, 91 and 56% for AZ225C, IMA1, IMA5, IMA6, Mix and Indy, respectively. Shoot N and P concentrations and contents were significantly affected by mycorrhizal inoculation at each harvest. Moreover, an intraspecific variability in P content was observed in *G. intraradices*. Regrowth ability and seeds dry weight were significantly increased by mycorrhizal inoculation (by 68% and 84%, respectively). Long term effect of AMF inoculation. *Z.mays* inoculated plants showed a high root colonization even after two years. *Z. mays* shoot biomass, N and P concentrations were not affected by AMF inoculation, whereas grain dry weight, numbers of ears and 1000 seeds dry weight showed significantly higher values in mycorrhizal plots than in controls. Interestingly, differences between mixed and single inocula were also observed (Mix > Single). Grain N e P contents were affected by mycorrhizal inoculation: host benefits calculated as N content were 31, 50, 38, 38, 69, 80% and calculated as P content were 44, 44, 22, 44, 56, 67% for AZ225C, IMA1, IMA5, IMA6, Mix and Indy, respectively.

Conclusions

The differential degree of host affinity found in the field may allow the selection of the most efficient plant – fungus combinations in terms of P and N uptake and growth parameters. The long term positive effects of mixed AMF inocula and of the indigenous population suggest the possibility of on-farm production of selected inoculum for low input and organic production systems.

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