



Species diversity and community composition of native arbuscular mycorrhizal fungi in apple roots are affected by site and orchard management



Alessandra Turrini^a, Monica Agnolucci^a, Michela Palla^a, Elisabetta Tomé^b, Massimo Tagliavini^b, Francesca Scandellari^b, Manuela Giovannetti^{a,*}

^a Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

^b Faculty of Science and Technology, Free University of Bolzano, Piazza Università 5, 39100 Bolzano, Italy

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are beneficial microorganisms which establish mutualistic symbioses with the roots of most food crops, improving plant performance, nutrient uptake and tolerance to biotic and abiotic stresses. A better understanding of the factors affecting AMF occurrence and diversity is fundamental to implement sustainable agricultural managements effectively profiting from beneficial plant symbionts. Here, we investigated AMF occurrence, diversity and community composition in the roots of apple trees from 21 orchards in South Tyrol, as affected by location, management (organic vs integrated) and altitude, by PCR cloning and sequencing and PCR-DGGE of partial 18S rRNA gene. The screening of 448 clones from 21 clone libraries allowed the identification of 6 native AMF at the species level: *Glomus indicum*, *Sclerocystis sinuosa*, *Funneliformis mosseae*, *Rhizoglomus irregulare*, *Septoglomus constrictus* and *Claroideoglomus lamellosum*. The most abundant genera were represented by *Glomus* (29.7% of the sequences), *Paraglomus* (19.4%), *Claroideoglomus* (17.2%), *Sclerocystis* (16.1%) and *Rhizoglomus* (12.3%). *Septoglomus*, *Diversispora* and *Funneliformis* sequences corresponded to less than 4% of total sequences. Although the degree of root colonization was unaffected by treatments, ANOSIM analysis of PCR-DGGE clusters revealed significant differences in apple root AMF diversity between sites and agricultural managements. Species richness was significantly higher in organically managed orchards than in integrated ones. Our findings provide insights into important factors affecting native AMF communities of apple trees, which could be exploited in sustainable fruit production systems, where beneficial soil biota boost biogeochemical cycles, energy fluxes and crop productivity.

1. Introduction

Soil microorganisms are essential elements of soil health, fertility and productivity in sustainable and organic farming systems, playing key roles in the completion of biogeochemical cycles and availability of mineral nutrients, carbon sequestration and soil structure improvement, pest and disease control (Pimentel et al., 1997). Arbuscular mycorrhizal (AM) fungi (AMF, Phylum Glomeromycota) are beneficial microorganisms establishing mutualistic symbioses with the roots of most food crops, including cereals, legumes and fruit plants, and improving plant performance, nutrient uptake and tolerance to biotic and abiotic stresses (Smith and Read, 2008). AMF are obligate biotrophs, obtaining sugars from their host plants while providing soil mineral nutrients – such as P, N, S, K, Ca, Fe, Cu and Zn – absorbed and translocated by means of large and ramified extraradical hyphal

networks that spread from plant roots into the surrounding soil (Giovannetti et al., 2015). Moreover, AMF deliver important agroecosystem services, such as soil aggregation and carbon sequestration (Gianinazzi et al., 2010), and are considered important soil biological indicators (Creamer et al., 2016; Stone et al., 2016). AMF enhance plant performance and fitness also through the synergistic action of beneficial mycorrhizosphere bacteria, i.e. strictly associated to mycorrhizal hyphae, colonised roots and spores, which display multifunctional activities, from antibiotic, siderophores and indole acetic acid production to P-solubilisation, phytate mineralization and N-fixation (Barea et al., 2002; Philippott et al., 2013; Agnolucci et al., 2015). Thus, AMF represent environmentally-friendly biofertilisers and biostimulants, as they reduce the need of chemical fertilizers and pesticides in sustainable food production systems (Rouphael et al., 2015). In addition, they contribute to the production of safe and high-quality food, positively

* Corresponding author.

E-mail address: manuela.giovannetti@unipi.it (M. Giovannetti).

affecting the synthesis of health-promoting secondary metabolites in food crops (Battini et al., 2016a,b).

In spite of all the potential benefits of the symbiosis, AMF role has been often marginalised in agriculture. In field crops, several agronomic practices, such as continuous monocultures, deep ploughing, intensive fertiliser and pesticide use can decrease AMF occurrence, activity and diversity, often leading to a reduction of AMF benefits to crop production and soil quality (Douds and Millner, 1999; Jansa et al., 2003; Oehl et al., 2004, 2005b; Brito et al., 2012; Avio et al., 2013). Moreover, in fruit production the soil is usually replanted with the same tree species short after the preceding trees are removed. In organic farming systems, thanks to the fact that synthetic fertilizers, herbicides and pesticides are not applied, AMF diversity, activity and abundance have been reported to be higher than in conventional systems, supporting the view that AMF play a fundamental functional role in the maintenance of soil fertility and crop production in low-input agroecosystems, compensating for the reduced use of agrochemicals (Mäder et al., 2002; Oehl et al., 2003, 2004; Gosling et al., 2006; Ryan and Tibbet, 2008; Mazzoncini et al., 2010; Verbruggen et al., 2010).

Different strategies have been devised to increase the mycorrhizal potential of soils in sustainable agriculture: among them, the inoculation with non-native AMF (Jeffries et al., 2003; Gianinazzi and Vosatka, 2004) has proved effective in improving root colonization, plant biomass production and P uptake (Lekberg and Koide, 2005). However, such an approach involves high costs of production and application when utilised on a large scale, and raises concerns about potential negative impacts of non-native invasive AMF inoculants on the composition and structure of native AMF communities, possibly leading to biodiversity losses (Schwartz et al., 2006). A different approach focuses on the enhancement of native AMF by means of mycotrophic crops able to maintain or increase native mycorrhizal potential of soils and root colonization and growth of the subsequent crops (Kabir and Koide, 2002; Karasawa and Takebe, 2012; Lehman et al., 2012; Njeru et al., 2015, 2014).

Little information is available on the impact of cultural practices on AMF occurrence, species richness and composition in apple crop production systems. Different orchard floor managements create different levels of soil disturbance and may therefore promote or depress AMF occurrence. Organic farming guidelines, for example, differently from conventional or integrated fruit production techniques (Kelderer, 2004), do not allow the use of chemical herbicides, but recommend, instead, the use of alternative techniques to manage the orchard floor by either superficial soil tillage, grass mowing or mulching. Similarly, while conventional and integrated management techniques allow the use of synthetic mineral fertilisers, organic farming guidelines recommend increasing soil fertility by enhancing soil organic matter. The use of straw mulches and compost have been reported to increase apple root colonisation, which was positively linked with soil pH and availability of P, K, Zn, Mn and C, suggesting that the relevant organic orchard floor management practices promoted functional AMF associations more effectively than conventional practices (Meyer et al., 2015). A recent work carried out using molecular methods showed that soil characteristics and farming systems affect AMF diversity and community composition in the roots of cultivated apple trees. In particular, a low soil available P content was associated with a higher AMF diversity in organically managed orchards, as compared with conventionally managed ones (Van Geel et al., 2015). In such a study, the native AMF colonizing apple roots were identified by 454-pyrosequencing of small subunit rRNA gene amplicons, with a taxonomic resolution at the family level. So far, the characterization of apple AMF communities at the species level has been performed only by conventional morphological methods, which show several shortfalls, mainly due to the use of spores extracted from the soil, whose integrity and quality may induce species misidentification (Cavallazzi et al., 2007; Miller et al., 1985; Purin et al., 2006).

Here we used two molecular methods, PCR-cloning and sequencing

and PCR denaturing gradient gel electrophoresis (PCR-DGGE) of partial 18S rRNA gene to characterize AMF communities of apple roots at genus and species levels. We investigated AMF occurrence and diversity in the root systems of apple trees cultivated under organic and integrated management from 21 orchards in the South Tyrol province (Italy), one of the most important apple production districts in Europe, with more than 18,000 ha acreage and approx. 1 million tons apples harvested yearly (Dalla Via and Mantinger, 2012). Our data provide knowledge of the factors which affect AMF colonization and shape the native AMF community composition in apple roots, allowing the implementation of effective sustainable management strategies that take advantage from beneficial plant symbionts.

2. Materials and methods

2.1. Soil and orchard management parameters

The research was carried out in South Tyrol (Northern Italy). In May 2013, 21 commercial apple (*Malus domestica* Borkh) orchards located either in the municipalities of Terlano (46°31' 59" N, 11°14' 47" E) or in that of Lagundo (46° 41' 0" N, 11° 8' 0" E) were randomly selected from a list provided by the local advisory service (The South Tyrolean Advisory Service for Fruit and Grape growing, Lana, Italy). This list included drip-irrigated apple orchards planted between 2007 and 2009, managed since planting following either the integrated fruit production (hereafter referred to as "integrated") or the organic farming (hereafter referred to as "organic") guidelines.

In the municipality of Lagundo 3 organic and 3 integrated orchards were selected at low (298–334 m a.s.l.) and at high altitude (591–660 m a.s.l.) while in the municipality of Terlano 6 organic and 3 integrated orchards were selected at a low altitude (ranging from 243 to 299 m a.s.l.). Orchards were classified according to the area (Te, Terano; La, Lagundo), management (O, Organic; I, Integrated) and altitude (L, Low altitude; H, High altitude) (Fig. 1).

The climate area is warm temperate according to the Koppen-Geiger classification (Kottek et al., 2006). Average (1993–2013) climatic parameters are reported in Table 1. Average annual and maximum temperatures at high elevation were approx. 2 °C lower than at low elevation, while average minimum temperature was approx. 1 °C lower. Soils in the area are mainly sandy loam or loamy sand and are classified as Calcaric Cambisol according to the FAO Soil Taxonomy (IUSS Working Group WRB, 2015) (Table 2).

The trees were always grafted on the same clonal rootstock (M9, T337 strain) and belonged to one of the following varieties: Gala, Golden Delicious, Pinova, Modì and Red Delicious. Orchards were uniform in terms of training systems (spindle bush) and planting distances (approx. 3 m between rows and 1 m between trees along the row). Orchard floor management always included the presence of grassed alleys between rows (2.0–2.3 m wide) that were mowed 3–4 times per year, while in the 0.7–1.0 m soil strip along the tree row, soil management differed between organic and integrated orchards (see below). The organic and integrated orchards differed both for the type of protection against aboveground pests and for soil management. In particular, in the organic orchards weed control was carried out through mechanical removal of weeds (approximately five times per year) and soil fertility was maintained only by the addition of organic fertilisers. In the integrated orchards, weed control in the soil strip under the trees was performed by spring and autumn application of glyphosate; mineral fertilizer supply was carried out according to the nutrient budget, considering expected yields of approximately 60 t ha⁻¹ and soil fertility, in the following ranges, 30–80 kg N, 19–28 kg P, 71–155 kg K.

2.2. Root, leaf and soil sampling

In each orchard, root and leaf were sampled from 6 randomly

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