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Functional identity has a stronger effect than diversity on mycorrhizal symbiosis and productivity of field grown organic tomato

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ABSTRACT

Beneficial soil biota, and in particular, arbuscular mycorrhizal fungi (AMF) are increasingly being recognized as key elements of organic and low-input agriculture where agrobiodiversity is central to enhanced crop production. However, the role of AMF in diversified organic systems, especially in field crops, is still poorly understood. A 3-year field experiment was carried out in Central Italy to investigate whether organic cropping systems that promote species and genetic diversity are more prone to mycorrhizal symbiosis increasing tomato growth, production and yield quality. Three tomato cultivars with varying genetic diversity were grown following four cover treatments: Indian mustard (*Brassica juncea* L. Czern.), hairy vetch (*Vicia villosa* Roth), a commercial mixture of seven cover crop species (Mix 7) and no-till fallow. Plants were either inoculated or not in nursery, with the two AMF isolates *Funneliformis mosseae* (IMA1) and *Rhizoglomus intraradices* (IMA6) used alone or mixed in a 1:1 volume ratio. On average, Mix 7 produced higher shoot dry matter (5.0 t ha^{-1}) than *V. villosa* (3.5 t ha^{-1}) or *B. juncea* (2.5 t ha^{-1}). Pre-transplant inoculation increased tomato root colonization at flowering and harvest compared to the non inoculated plants (31.8 *vs* 23.6%) and cv. Rio Grande was on average the best colonized. The mean fresh weight of marketable fruits was 18.4, 28.0 and 28.6 t ha^{-1} for cvs. Rio Grande, Roma and Perfect Peel, respectively. Cover crops inconsistently affected tomato marketable fruit production in year 1, while in years 2 and 3, *Vicia villosa* and Mix 7 showed the best effect respectively. In year 3, among the pre-inoculated plants those treated with isolate IMA6 showed a higher production of marketable fruit number m^{-2} (56.7) than those inoculated either with IMA1 (51.5) or the mixed inocula (52.1). Most fruit quality parameters were affected by tomato genotype. This study shows that while increased agrobiodiversity is important to increase agroecosystem resilience, AMF, crop and cover crop functional identity may be more important than diversity *per se* to promote mycorrhizal symbiosis and productivity of field grown organic tomato.

1. Introduction

Organic agriculture has risen considerably in the last two decades motivated by burgeoning consumer demand for healthier food and the need to conserve the environment and maintain biodiversity (Willer and Kilcher, 2012). Soil fertility and crop production in organic agriculture are maintained through diverse crop rotations and enhanced nutrient cycling where soil biota play a critical role. Arbuscular mycorrhizal fungi (AMF) are an important group of soil microorganisms living in symbiosis with the majority of cultivated crops. AMF enhance crop nutrition and protection through a large network of extraradical hyphae spreading from colonized plant host roots to the surrounding environment (Avio et al., 2006; Oruru and Njeru, 2016). The composition and function of AMF communities is expected to vary upon crop genotype, agronomic management practices and soil conditions (Njeru et al., 2014; Turrini et al., 2016). Among the former, the importance of crop genotype is gaining pace. Recent evidence shows that mod-

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ern hybrids, bred to exploit high input conditions, may be less prone to mycorrhizal symbiosis compared to older crop varieties and landraces (Lehmann et al., 2012; Singh et al., 2012).

Cover crops are important in organic cropping systems where they are used as green manure, dead or living mulch. The role of cover crops in supplying essential plant nutrients, suppression of plant diseases, weeds and parasitic nematodes is well known (Clark, 2007; Moonen and Bàrberi, 2006). Indeed, cover crops are crucial in maintaining and restoring soil biodiversity, especially of AMF which are obligate mutualists (Kabir and Koide, 2000; Kabir et al., 2008). However, some cover crops, in particular members of the *Brassicaeae* family, are non-AMF hosts and may be detrimental to AMF although they provide other agroecosystem services (AES) e.g. reduction of nitrate leaching, and suppression of weeds and soil borne pathogens (Weil and Kremen, 2007; White and Weil, 2010). While there is growing interest in the use of cover crops, farmers often choose to grow single cover crop species depending on their preferences and on prevailing environmental conditions (Zibilske and Makus, 2009). Since different cover crops generally provide varying AES and perform differently based on agro-climatic conditions, we can hypothesize that increased cover crop diversity (i.e. mixtures) may foster production as well as resilience and stability in organic farming systems.

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop that readily benefits from mycorrhizal symbiosis through increased nutritional uptake, general plant health (Cavagnaro et al., 2006), production and yield quality (Giovannetti et al., 2011; Ortas, 2012). Moreover, AMF colonization in tomato induces resistance to diseases (Fritz et al., 2006; Song et al., 2015) and nematodes (Vos et al., 2012) as well as tolerance to abiotic stresses, such as drought (Ruiz-Lozano et al., 2016) and salinity (Al-Karaki, 2006).

To increase AMF symbiosis in tomato and other horticultural crops, plants can be pre-inoculated with exotic AMF isolates at nursery, where the mycorrhizal inoculum is mixed with a sterile substrate used for seedling preparation. In this case, AMF colonization is achieved at a juvenile stage and in absence of competition from indigenous fungal endophytes (Jeffries et al., 2003). After transplanting, the AMF inoculated plants have higher root colonization, which may increase AMF symbiosis in the field, enhancing growth and production. Although the potential of AMF to improve tomato production and quality has been previously demonstrated in greenhouse experiments (Subramanian et al., 2006) their application under field conditions remains relatively limited. Field experiments on AMF are challenging, e.g. in establishing non mycorrhizal control treatments and manipulating AMF on a large scale (Martinez and Johnson, 2010).

Empirical evidence from previous studies suggested that AMF colonization and responsiveness vary among crop cultivars, depending on age, origin and type of cultivar (An et al., 2010; Singh et al., 2012; Turrini et al., 2015). Choice of crop genotype, preceding cover crop and inoculated fungal isolate are expected to influence AMF colonization. The diversity level of these factors may affect mycorrhizal symbiosis and expression of related AES, upon effects driven either by functional identity (i.e. choosing the right species), functional composition (i.e. heterogeneity between species) or functional diversity (i.e. heterogeneity within species) (Costanzo and Bàrberi, 2014).

In this study, we hypothesized that cropping systems based on higher diversity of biological components (cover crops, cash crop and fungal symbionts) at the genetic or species level would give better growth and production of field grown processing tomato. Therefore, an organically managed field study based on increasing levels of genetic and species agrobiodiversity was carried out to investigate whether the ability of processing tomato to associate with AMF, form effective symbiosis and increase yield and produce quality is better with use of (a) open pollinated varieties *vs* a modern hybrid; (b) a cover crop species mixture *vs* single cover crop species; (c) pre-transplant AMF inoculation with two AMF strains *vs* one.

2. Materials and methods

2.1. Experimental site and soil characteristics

Field experiments were carried out in three nearby fields (one per year) at CIRAA (Interdepartmental Centre for Agri-environmental Research "Enrico Avanzi", University of Pisa, S. Piero a Grado, latitude 43°40′ N, longitude 10° 20′ E), in the coastal plain of Tuscany, central Italy. The soil in the 0–30 cm layer is a sandy-loam with physical and chemical properties ranging from; clay 11.1–21.1%, silt 16.9–21.5%, sand 57.4–72.0%, pH (H_2O) 6.5–7.9, Organic C 2.2–3.5%, total N (Kjedahl) 1.1–2.8 g kg^{-1}, P (ppm, Olsen) 3.1–4.6. The climatic conditions are typical of Mediterranean areas, with rainfall mostly concentrated in autumn (October to December) and spring (March to April) (see graphs in Fig. A.1 and Fig. A.2 in Supplementary Material).

2.2. Experimental design

Field experiments involving a cover crop-tomato sequence and maintained under certified EU organic standards were carried out from September 2010 to September 2013. The experiments were laid as split-split-plot design with three blocks (replicates). Main plots consisted of four cover crop treatments, namely *Brassica juncea* (L.) Czern. cv. ISCI 20 (Indian mustard), *Vicia villosa* Roth cv. Latigo (hairy vetch), a mix of seven species (hereafter 'Mix 7') and a no-till fallow with natural vegetation (hereafter 'Control'). The Mix 7 treatment, supplied as a commercial seed mixture by Arcoiris s.r.l (Modena, Italy), included *Fagopyrum esculentum* Moench (buckwheat), *Lupinus albus* L. (white lupin), *Phacelia tanacetifolia* Benth. (lacy phacelia), *Pisum sativum* L. (common pea), *Trifolium alexandrinum* L. (berseem clover), *Trifolium incarnatum* L. (crimson clover) and *V. villosa.* Subplots included a commercial hybrid (cv. Perfect Peel) and two open pollinated varieties (cv. Roma and cv. Rio Grande), all from Arcoiris s.r.l. (Modena, Italy). Cvs. Roma and Rio Grande are old varieties, while Perfect Peel is a modern hybrid commonly grown for industrial use in Italy (www.sementi.it accessed on 4 April 2016). Sub-sub-plots hosted four mycorrhizal treatments, including *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov., isolate IMA1, *Rhizoglomus intraradices* Schenck & Smith, isolate IMA6, IMA1 + IMA6 (1:1 v/v%) and a mock treatment used as non inoculated control (hereafter Mock). The experimental trial comprised 144 sub–sub plots each measuring 3 × 5 m in the first year 3 × 4 m in the second year and 3 × 3.5 m in the third year.

2.3. Cover crop management

The cover crops were sown on 18 October 2010 at a seeding rate of 9 kg ha^{-1} (*B. juncea*), 100 kg ha^{-1} (*V. villosa*), 50 kg ha^{-1} (Mix 7). In the second and third year, cover crops were sown on 19 October 2011 and 15 October 2012 at an increased seed rate of 12 kg ha^{-1} (*B. juncea*), 120 kg ha^{-1} (*V. villosa*), 65 kg ha^{-1} (Mix 7) to enhance establishment. The Control plots were left fallow and weeds were not controlled, like in all the other cover crop treatments. The cover crops and weeds were then mown and immediately incorporated into the soil by disc harrowing. Seeding beds were then raised and black plastic mulch film and drip irrigation tapes were laid onto the soil.