DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN HORTICULTURAL PRODUCTION SYSTEMS OF SOUTHERN CHILE

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ABSTRACT

The diversity of arbuscular mycorrhizal (AM) fungi in six *Capsicum annuum* or *Lycopersicum esculentum* L. horticultural production systems of small farmers, and of two wheat agrosystems was comparatively investigated in Southern Chile (La Araucanía). Soils in this region are mostly originated by volcanic ashes which are characterized by high organic matter content and high P-fixing capacity. Arbuscular mycorrhizal fungal symbioses are playing a key role for P uptake by horticultural crops grown there. The objective of this study was to determine AM fungal communities in cropping systems and to identify soil factors affecting their frequency and diversity. Of the totally 32 AM fungal species identified, 5 to 21 AM fungi species were found in horticultural locations and 8 to 11 AM fungi species in conventional tillage (CT) and no-tillage (NT) agroecosystems. No relationships on AM fungal diversity with soil factors were found. In wheat based agrosystems fungal diversity was somewhat lower under CT than NT whereas no such relationships between diversity and intensity of land use could be generated from horticultural systems. It is concluded that it will be advisable for farmers to inoculate their horticultural crops with selected mycorrhizal inoculants during the nursery stage, as it cannot be predicted from the soil conditions whether the native AM fungal community is sufficient to sustain a stable horticultural production in the region.

Keywords: Glomeromycetes, pepper, lettuce, agro-ecosystems.

INTRODUCTION

Soil microbiota plays a fundamental role for the productivity and stability of horticultural and agroecosystems in the volcanic soils of Southern Chile (Castillo *et al*., 2006b; 2009). Within this microbiota arbuscular mycorrhizal (AM) fungi stand out because they are so important for the phosphate (P) nutrition of plants in the acidic Andisols (Borie *et al*., 2010). They establish a mutualistic symbiosis with most of terrestrial plants and this type of mycorrhiza is by far the most important worldwide. It is said that it occurs with more than 60 % of the species of the plant kingdom (Trappe, 1987). Fungal species involved in the formation of AM belong to the Glomeromycota (Schüssler *et al.*, 2001).

Among the factors influencing the AM fungal community dynamics and associations with plants, agricultural practices may be considered to be the most important. Tillage, crop rotation sequences, and crop management systems have been reported as critical factors affecting the development, activity and diversity of AM fungi (Oehl *et al.*, 2003; 2005). Mycorrhizal propagules can survive in the soil as spores which appear to be long-term structures of different ages, states of dormancy and germination periods and they constitute an inoculum source persisting for many years (Smith and Read, 2009). Hence, these fungal propagules represent the native mycorrhizal inoculum potential for locally grown crops. The fungal species naturally present in agrosystems may be decisive for the productivity of crops in the P-deficient soils of Southern Chile. The main purpose of this study was to investigate the occurrence of AM fungi species in some horticultural ecosystems in some soils of Southern Chile, and to determine whether there is any relation between their occurrence and soil factors. As for comparison, no till and conventional wheat agrosystems were included in the study.

MATERIALS AND METHODS

Two agroecosystems were selected for the study in the Araucanía Region of Chile: horticultural systems habitually cropped by local farmers with vegetables such as pepper and tomato, and a wheat based cropping system. Soil samples were taken from six districts where vegetables are cultivated: Angol, An (37º48`; 73º41`); Lumaco, Lu (38º40`; 73º58`); Purén, Pu (38º09`; 73º00`); Los Sauces, Sau (38º15`; 72º50`); Vilcún, Vil (38º40`;72º14`); Lobería, Lo (38º39`; 73º29`), and from wheat cultivated under no tillage (NT) and conventional tillage (CT) at Pumalal (38º40`; 72º31`).

In Angol, Lumaco, Purén, and Los Sauces the soils were cultivated with chili pepper (*Capsicum annuum* L.) locally named "Cacho de Cabra", for many decades. In Vilcún soil, tomato was cultivated and at Lobería the soil was under fallow, after a previous vegetable cropping (Table 1). Four soil samples from the 0-10 cm horizon were taken from 10 m^2 plots from within the planting rows and between the rows. There were 4 replicated plots per location. Soil samples from each plot were bulked to give four replicates per ecosystem; soil samples were brought to the lab and stored in a freezer until analyses for separation of spores and the identification of AM fungi species, and for soil analyses.

In each ecosystem the following soil chemical parameters were determined: a) soil pH which was measured by glass electrode in a 1:2.5 soil: water suspension; b) available phosphorus which was determined after extraction with a solution of 0.5 M $NaHCO_3$ at pH 8.5 (Olsen and Sommers, 1982; and c) soil organic matter (SOM), was measured according to Walkley and Black (1934). Such soil characteristics appear in Table 1.

Spores of AM fungi were extracted and separated from the soil using the wet-sieving and decanting procedure described by Sieverding (1991). Spores were isolated under a stereomicroscope and fixed in polyvinyl alcohol-lactic acid-glycerol (PVLG), and a mixture of PVLG and Melzer`s reagent (Brundrett *et al.*, 1996) to obtain permanent specimen. For the taxonomic classification main morphological spore characteristics such as colour, size, type and number of spore walls, and the morphology of the subtending hypha at the point of spore attachment were observed under a high-

Table 1. Soil chemical characteristics in the horticultural and agroecosystems of Southern Chile investigated.

Community	Time of sampling	Vegetation at sampling	pH	Olsen-P (mg kg^{-1})	SOM (%)
Angol	April 2007	Chili pepper harvest, mature fruits	7.3	106	7.0
Los Sauces	April 2007	Tomato harvest, mature fruits	5.5	10	6.0
Lumaco	April 2007	Chili pepper harvest, mature fruits	5.9	38	6.0
Purén	October 2006	Fallow after harvest of chili pepper	5.9	48	5.0
Lobería	December 2007	Fallow; vegetable crop in the preceeding year	6.1	4.0	1.8
Vilcún	December 2007	Host tomato, trial 4 months old	6.1	5.5	8.2
Pumalal CT	April 2003	Fallow after wheat harvest	5.8	22	8.2
Pumalal NT	April 2003	Fallow after wheat harvest	5.8	18	8.2

power light microscope at 100x and 400x magnification. Species identification was carried out using the keys and instructions given by Schenck and Pérez (1990) and INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi, see internet homepage: www.invam.caf.wvu.edu). All isolated specimen were deposited at the Laboratory of Plant Nutrition of the Universidad de La Frontera, Temuco, Chile.

RESULTS AND DISCUSSION

In total, 32 species of Glomeromycota were found in the six horticultural and the two wheat crop systems studied (Table 2).

Of the known species, 17 of them were previously not described (Castillo *et al.*, 2006a) from Southern Chile: *Acaulospora alpina, Ac. laevis, Ac. longula, Ac. paulina, Ac. undulata, Glomus aggregatum, Gl. claroideum, Gl. clavisporum, Gl. fasciculatum, Gl. geosporum, Gl. monosporum, Gl. mosseae, Gl. pallidum, Gl. vesiculiferum, Gl. versiforme, Pacispora dominikii*, and *Paraglomus occultum*. The number of species was highly variable among the horticultural production sites ranging from 5 species in Purén to 21 species in Vilcún, and was less variable in wheat production systems with 8 species under CT to 11 species under NT.

Only 6 of the 32 AM fungal species were found at 50 % or more of the districts, so that their geographic distribution was somewhat higher than the other species: *Archaeospora trappei*, *Gl. coronatum*, *Gl. diaphanum*, *Gl. etunicatum*, *Gl. intraradices*, and *Gl. versiforme*. This may indicate that these species are fit to survive and form spores under very diverse conditions. Therefore, these 6 fungal species can be considered ecological generalists (Castillo *et al*., 2006a). In this sense, *Gl. etunicatum* is known as a generalist from European studies (Oehl *et al.*, 2004), and from evergreen forests, deciduous forests and grassland ecosystems of Southern Chile (Castillo *et al.*, 2006a). On the other hand, *Gl. versiforme* only is a generalist species in horticultural ecosystem; whilst some other species like *Gl. invermaium*, and *Scutellospora calospora*, were only observed in the wheat production system.

Table 2. Genera and species of the Glomeromycota found in the horticultural systems in Angol (An), Lumaco (Lu), Purén (Pu), Los Sauces (Sau), Vilcún (Vil), Lobería (LO), and agroecosystems at Pumalal under no-tillage (NT) and conventional tillage (CT).

Genus	Species name	Horticultural system*						Agroec.	
		An	Lu	Pu	Sau	Vil	Lo	NT	CT
Acaulospora	*alpina* Oehl, Sykorova and Sieverd.		x			x			
	laevis Gerd. and Trappe					x			
	longula Spain and N.C. Schenck					x	x		
	paulina Blaszk.			x		x			
	spinosa C. Walker and Trappe		x			x			x
	thomii Blaszk.			x				x	
	undulata Sieverd.		x			x			
	sp. (unknown)					x			
Archaeospora	*trappei* J.B. Morton and D. Redecker emend Spain	x	x		x	x		x	
Diversispora	*spurca* C. Walker and A. Schüssler				x		x	x	
Gigaspora	sp.(unknown)	x							
Glomus	*aggregatum* N. C. Schenck and Smith					x			
	claroideum N.C. Schenck and G.S. Sm. emend C. Walker andVestberg	x	x			x			
	clavisporum R.T. Almeida and N.C. Schenck	x							
	coronatum Giovann.		x			x		x	x
	diaphanum J.B. Morton and C. Walker			x		x		x	x
	etunicatum W.N. Becker and Gerd.	x	x		x		x	x	x
	fasciculatum Gerd. and Trappe emend. C. Walker and Koske		x			x	x		
	geosporum C. Walker				x				
	intraradices N.C. Schenck and G.S. Sm.	x		x			x	x	x
	invermaium I.R. Hall							x	x
	macrocarpum Tul. and C. Tul.		x					x	
	monosporum Gerd. and Trappe					x			
	mosseae Gerd. and Trappe		x		x	x			
	pallidum Hall	x	x			x	x		
	vesiculiferum Gerd. andTrappe		x						
	versiforme Berch		x	x	x	x	x		
Pacispora	*dominikii* Oehl and Sieverd.					x	x		
Paraglomus	*occultum* J.B. Morton and D. Redecker		x		x	x			
Scutellospora	*calospora* C. Walker and F.E. Sanders							x	x
	dipurpurescens J.B. Morton and Koske					x		x	x
	sp. (potentially *Cetraspora*)					x			
Total species		7	14	5	7	21	8	11	8

* x indicates presence of arbuscular mycorrhizal fungal species

Some species appear to be not common, such as five species isolated at Vilcún, like *A. laevis*, an unknown *Acaulospora* sp., *Gl. aggregatum*, *Gl. monosporum*, and a *Scutellospora* sp.; two species at Angol: a *Gigaspora* sp. and *Gl. clavisporum*; one in Los Sauces: *Gl. geosporum* ; whilst at Lumaco, a species similar to *Gl. vesiculiferum* was identified. All these AM fungal species were only found once. Four AM fungal species were identified only at genera level (Table 2) originating from the districts of Vilcún and Angol.

In horticultural system, Lumaco and Vilcún soils presented relatively high diversity of AM fungi, and in the wheat cropping systems, higher species numbers were found under NT than CT. The function and importance of each of the different AM fungal species in each ecosystem still remains unknown.

In both ecosystems, the horticultural and the wheat cropping systems, *Glomus* spp. represented each about 60 % of all AM fungal species found clearly the majority of the AMF community (Figure 1). It is not surprising that most of AM fungi spores owned to the *Glomus* genus because this is the prevailing genus in agricultural soils among the AM species described so far (Jansa *et al.*, 2003), and also in Chile, in a wheat-oat crop rotation with the application of different tillage systems in an Ultisol most species belonged to the genus *Glomus* (Castillo *et al.*, 2006b). Similar results were obtained from European grasslands (Oehl *et al.*, 2004).

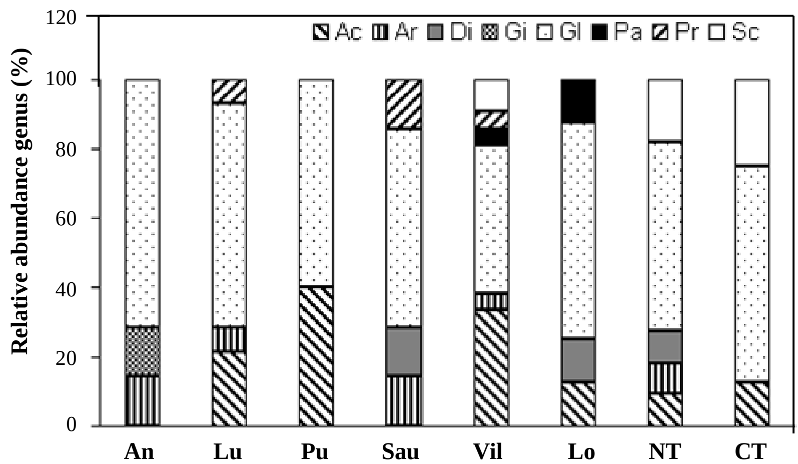

Figure 1. Relative abundance of fungal species for each genus (number of all fungal species identified at each site set to 100%) in agrosystems investigated of Southern Chile. Districts are: An: Angol; Lu: Lumaco; Pu: Purén; Sau: Los Sauces; Vil: Vilcún; Lo: Lobería; NT: no-tillage; CT: conventional tillage. Fungal genera found were: Ac: *Acaulospora*; Ar: *Archaeospora*; Di: *Diversispora*; Gi: *Gigaspora*; Gl: *Glomus*; Pa: *Pacispora*; Pr: *Paraglomus*; Sc: *Scutellospora*.

No correlation was found between the species richness, or occurrence of specific fungal species and the soil characteristics like organic matter content, P content or soil pH. Hence, from these parameters it is not predictable which fungal species might occur at a specific site. Horticultural systems in the region are often fertilized with high amounts or in contrast with reduced amounts of fertilizers depending on the economic situation of the small farmers. Very high available P contents of soils (e.g. Angol) may be an indicator for high organic or chemical fertilizer inputs. It is not clear from the current investigation whether such high inputs have a dramatic negative effect on the appearance of different fungal species as for example was demonstrated from European agricultural monocropping systems (Oehl *et al.*, 2003). Deeper studies could show such effects, but at this time we conclude that the bio-diversity in small farm horticultural systems in the Araucanía region is highly variable and not predictable from soil characteristics. This indeed may call for active management of mycorrhiza in horticultural systems by inoculation with selected effective mycorrhizal inoculants. Because the farmer will not know what kind of AM fungi species in which concentration he has in his field, he will produce a horticultural crop with less risk when inoculating his crop with selected mycorrhizal fungi at the nursery stage. Mycorrhizal seedlings are then transplanted to the field which will likely give a more secure production. Unfortunately, such mycorrhizal inocula are not yet available in the region but recent research has shown that inoculum production at large scale is possible in Chile (Castillo *et al.*, 2009). It is now up to commercial companies to make such inocula available.

CONCLUSIONS

The diversity of AM fungal species is highly variable in chilli pepper and tomato based horticultural production systems of small farmers in the Araucanía region of Chile. There is no relationship between the most common soil parameters with such diversity, and the occurrence or absence of AM fungi species is likely to depend on the divers agronomic historic inputs, that farmer apply to horticultural crops. Thus, because most of the horticultural crops depend obligatorily for growth on mycorrhizae, in the soils of the region, it is advisable to inoculate the horticultural crops with selected highly effective mycorrhizal inoculants at the nursery stage especially in organic agriculture. This will likely give farmers a more secure and sustainable production than to depend in crop production on unknown natural AM fungi communities.

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