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ORIGINAL PAPER

Agricultural Managements Influence the Diversity of Arbuscular Mycorrhizal Fungi in Vineyards from Chilean Mediterranean Climate Ecosystems

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Abstract

Purpose Grapevine (*Vitis vinifera* L.) is a relevant crop, which is associated to arbuscular mycorrhizal fungi (AMF) that are influenced by agricultural practices. The hypothesis of this study is that organic/biodynamic management stimulates grapevine mycorrhizal colonisation and increases AMF diversity in Chilean vineyards. The aim of this study was to determine the influence of agricultural management on AMF association and AMF diversity in Chilean vineyards.

Methods Mycorrhizal colonisation of grapevine roots from organic/biodynamic and conventional vineyards in Northern (Elqui Valley), Central (Casablanca and Cachapoal Valleys), and Southern Chile (Maule and Itata Valleys), was determined under a microscope. AMF diversity was analysed by morphological, and molecular characterisation of spores through SSU-ITS-LSU rRNA region sequence analyses.

Results AMF colonisation of grapevine roots was influenced by vineyard management independent of the season. Higher mycorrhizal colonisation was detected in organic/biodynamic grapevine soils (20 – 35%), compared with conventional soils (6 – 31%). Twelve AMF species were identified in vineyards, belonging to five Glomeromycota families. Interestingly, organic/biodynamic vineyards showed higher AMF diversity. The three predominant morphotypes were *Funneliformis verruculosum* (GL1), *Septoglomus* sp. (GL4) and *Septoglomus constrictum* (GL5). Molecular analyses of AMF spores highlighted the occurrence of *Septoglomus, Acaulospora, Pacispora* and *Cetraspora* genera in vineyards.

Conclusions In this study, AMF diversity in Chilean vineyards is described for the first time. The diversity of AMF in vineyards in Chile was higher than the diversity reported in other wine-producing ecosystems. The understanding of agricultural practices on AMF activity and diversity may be crucial to improve the vineyard management.

Keywords Arbuscular mycorrhizal fungi · *Glomeromycota* · *Vitis vinifera* · Chilean vineyards · *Septoglomus* · Organic agriculture

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1 Introduction

Vineyards are important in the Mediterranean climate region of Chile, covering 136,289 ha (Ribera-Fonseca et al. 2023). Grapevine represents a market of 2.1 billion dollars in wine exports, placing Chile as the fourth main wine exporter, with 6% of the world market (OIV 2018). In vineyards, different factors such as climate, agricultural management, soil, geography, landscape characteristics and human activities influence grapevine development, health, and productivity. Microbial biodiversity is one of the key elements of functional agroecosystems, playing an important role in vineyards (Balvanera et al. 2006; Gilbert et al. 2014; Hector and Bagchi 2007; Schreiner 2020). Climate change is affecting agriculture worldwide and in Chile, where increases in drought, salinity, extreme temperatures, and the emergence of novel phytopathogens have been observed (Aguilera et al. 2022; Larach et al. 2022; Valenzuela et al. 2021; Vasconez et al. 2020; Vega-Celedón et al. 2021). Therefore, to counteract the increasing abiotic and biotic stresses of food crops, diverse strategies including organic/biodynamic management, inoculation of native beneficial microorganisms, and plant grafting have been applied (Aguilera et al. 2022; Alfaro-Quezada et al. 2023; Álvarez-Hubert et al. 2024; Carvajal et al. 2023; Larach et al. 2024; Olivera et al. 2021; Vega-Celedón et al. 2021).

The rhizosphere, the narrow zone of soil that surrounds and is influenced by plant roots, has high microbial activity and is one of the most dynamic interfaces on Earth (Philippot et al. 2013; Vega-Celedón et al. 2021). Within rhizosphere microorganisms, arbuscular mycorrhizal fungi (AMF) from phylum Glomeromycota are obligate symbionts, beneficial to a high number of plants including most food crops. AMF increase absorption of nutrients and water, and promote defences and higher resistance to phytopathogens (Cameron et al. 2013; Schüβler et al. 2001; Smith and Read 2008; Tedersoo et al. 2018). AMF modify secondary metabolism in plants, enhancing the production of terpenoids and phenolic compounds, which increase food nutraceutical properties (Agnolucci et al. 2020; Avio et al. 2018; Kapoor et al. 2017; Velásquez et al. 2020a, 2020b). In recent reports, the enhancement of volatile organic compounds, polyphenols, flavonoids, flavonols, and anthocyanins has been demonstrated in roots and leaves of mycorrhizal grapevine plants (Gabriele et al. 2016; Krishna et al. 2005; Velásquez et al. 2020a, b).

In agricultural systems, agrochemicals may affect microbial and mycorrhizal communities (Hernández et al. 2011; Oehl et al. 2017). Decrease of AMF diversity negatively affects plant performance, as soil ecosystem processes regulated by microbiota are disrupted (Delgado-Baquerizo et al. 2020; Gianinazzi et al. 2010). Organic or agroecological practices that incorporate cover crops, polycultures, and minimize the use of agrochemicals and tillage, promote the establishment of a more diverse AMF community (Säle et al. 2015; Turrini et al. 2016, 2017a, b; Verbruggen et al. 2010; Verbruggen and Toby Kiers 2010). In vineyards, plant nutrition directly influences wine quality. The use of high levels of fertilisers reduces AMF colonisation and propagules, affecting the abilities of the fungi to explore microaggregates and increase water and nutrient uptake (Schreiner 2005). Tillage destroys AMF mycelium network, although some species can tolerate this disturbance. In vineyards and other crops, using cover crops fosters higher AMF diversity and abundance, benefiting the plants (Oehl and Koch 2018). It has been reported that AMF are diverse in the forests surrounding vineyards and that AMF diversity could be linked to the diversity of native plants, which should be studied when incorporating cover crops in the vineyards (Holland et al. 2016).

AMF diversity in vineyards of different countries has been reported, indicating a wide dependence on AMF symbiosis (Balestrini et al. 2010; Holland et al. 2016; Likar et al. 2013; Schreiner 2020; Schreiner and Mihara 2009). However, only specific wine growing regions in the world have been yet analysed. Therefore, studies of mycorrhizal communities in different geographical areas should be encouraged (Bouffaud et al. 2016; Likar et al. 2013; Massa et al. 2020). The presence and diversity of AMF in Chilean vineyards have not been reported yet. However, microbial biodiversity associated to grapevines have been investigated in vineyards in Chile, indicating a high diversity of bacteria and free–living fungi (Castañeda et al. 2015; Castañeda and Barbosa 2017; Gilbert et al. 2014; Miura et al. 2017, 2019).

In Chile, the potential benefits of including native flora in vineyards have been reported, as it fosters biodiversity and acts as a reservoir of microorganisms (Castañeda and Barbosa 2017; Jara et al. 2016; Viers et al. 2013; Miura et al. 2019; Vega-Celedón et al. 2021). The hypothesis of this study is that organic management stimulates the mycorrhizal colonisation of grapevine and increases the AMF diversity in Chilean vineyards. The aims of this study were to determine the influence of plant management on AMF colonization of grapevine roots and to characterise AMF diversity in Chilean vineyards.

2 Materials and Methods

2.1 Soil Sampling and Characterisation

This study was conducted at ten vineyards that were > 20 years old, distributed in five zones: Elqui,

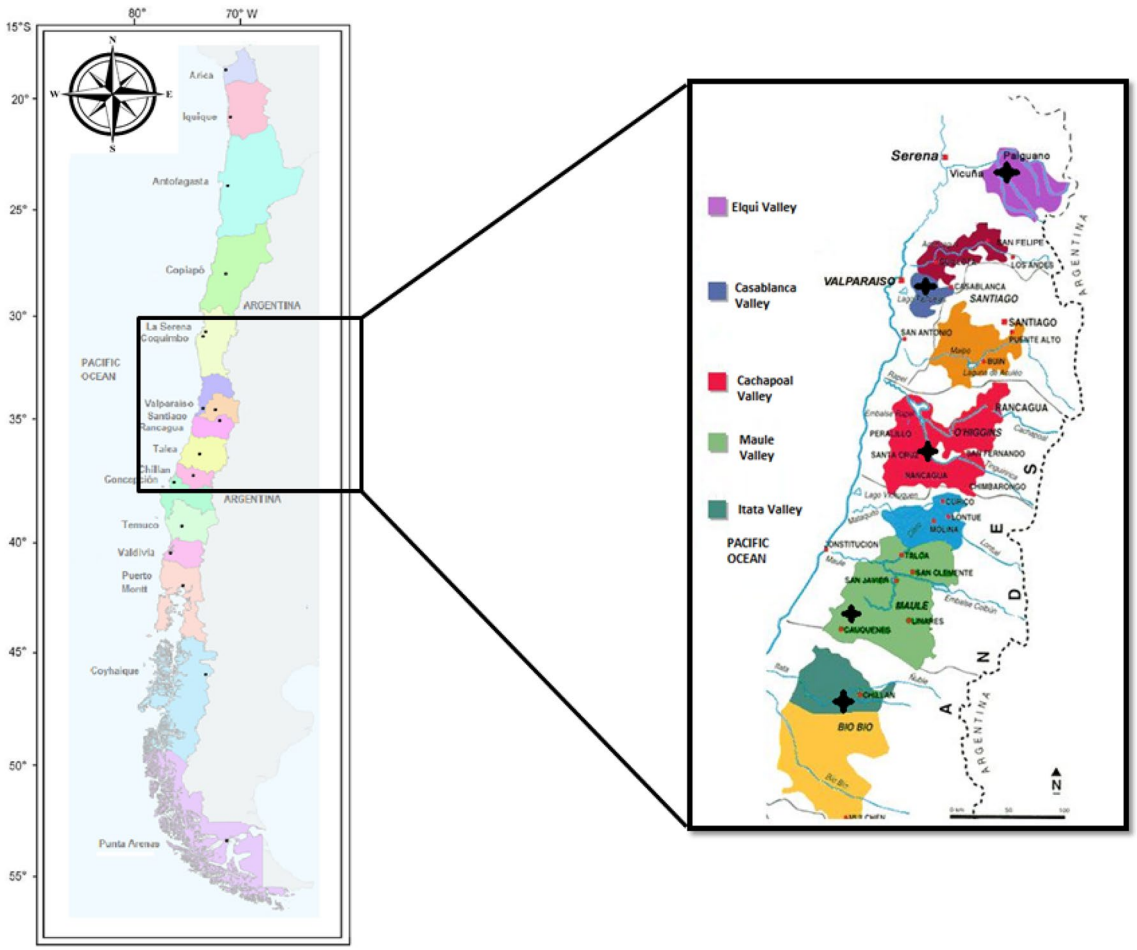

Fig. 1 Location of vineyards in Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys of the Chilean Mediterranean climate region. The five valleys are shown. Location of vineyards with organic/biodynamic and conventional management are labelled with a cross

Casablanca, Cachapoal, Maule and Itata valleys in the Chilean Mediterranean-type ecosystem (Fig. 1). In each valley a conventional vineyard and an organic or biodynamic vineyard from *Vitis vinifera* L. variety Cabernet Sauvignon were sampled during winter (July 2015) and summer (January 2016). The conventional vineyards were managed with practices that reflect the commercial standards from Chilean production, applying pesticides, and fertilisers. The organic or biodynamic vineyards were managed with practices that reflect the main international certification standards, without application of agrochemicals, and using cover crops and compost (Murphy et al. 2022). The vineyards did not use AMF inoculation.

Root and soil samples for the determination of mycorrhizal colonisation and fungal spore characterisation were collected in the planting row of grapes plants in each vineyard with a 10 cm diameter soil core 30 cm deep (500 g). For the identification of vineyard roots, the root system was examined according to its morphoanatomical characteristics. Ten replicate samples were collected along a transect across each vineyard, covering most of the vineyard surface. The samples were placed in polyethylene bags and stored at 4 ºC until processed.

Soil pH was determined in a 1:2.5 (w/v) soil water ratio. Total organic C was quantified by the Walkley–Black method (Nelson and Sommers 1996). Total N was determined by Kjeldahl digestion (Bremner 1996). The available Olsen P was measured by extracting soil with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen and Sommers 1982). Exchangeable K was determined using the ammonium nitrate method (Mehlich 1984).

2.2 Analysis of Mycorrhizal Colonisation in Grapevine Roots

Roots were carefully washed in tap water to remove soil particles and organic matter. Thin healthy roots were cut into small pieces (1 cm), cleaned, and stained (Brundrett et al. 1996). Twenty root segments (~ 1 cm) per plant (n = 10) were used to determine mycorrhizal colonisation according

to Trouvelot et al. (1986). The mycorrhizal colonisation was determined under a dissecting microscope (Zeiss, Germany) at 40 × magnification. Every root segment was assigned to a relative category of mycorrhizal colonisation from 0 (0% colonisation) to five (> 95% colonisation). The colonisation percentage was then calculated as follows:

$$\% \text{ M } = (n_1 + n_2 \times 5 + n_3 \times 30 + n_4 \times 70 + n_5 \times 95)/ \text{ n total x } 100$$

where % M is symmetrical in the 5 – 95% range, n total is the number of observed segments, while n_1 to n_5 represents the number of segments categorised as 1 to 5, respectively. The AM colonisation percentage was obtained for each plant (Trouvelot et al. 1986).

Selected stained root segments were mounted on glass slides in lactoglycerol to observe intraradical fungal structure with a Polyvar light microscope (Zeiss, Jena, Germany) at 25 × or 40 × magnifications.

2.3 Fungal Spore Extraction and Identification

Spores and sporocarps of AMF were extracted from duplicate sieving of 25 g of each sample (n = 10), by wet–sieving and decanting, through a set of nested sieves with 400, 250, 160 and 45 µm mesh (Gerdemann and Nicolson 1963). After sieving, the material obtained from the 160 and 45 µm sieves was centrifuged at 448 g for 2 min in a water/sucrose solution density gradient. The supernatant was filtered through a 45 µm sieve, washed with tap water, and the trapped material, largely containing spores, spore clusters and sporocarps, was examined under a dissecting microscope (Leica, Wetzlar, Germany) at magnifications up to 50 ×. Only intact healthy spores were counted. Spores were separated according to their morphology and colour, placed in Eppendorf tubes, submitted to sonication in a B–1210 cleaner (Branson Ultrasonics, Soest, The Netherlands), washed in sterile distilled water, mounted on microscope slides in polyvinyl alcohol lactoglycerol (PVLG) and examined under a Polyvar light microscope equipped with Nomarski differential interference contact optics (Reichert–Young, Vienna, Austria). For taxonomical identification based on morphology, 20 spores were mounted both in PVLG and in PVLG + Melzer's reagent (1:1, v/v) medium (Gerdemann and Trappe 1974). Qualitative spore traits (spore shape, colour and size, spore wall ornamentation, wall structure and shape, colour, and size of the subtending hypha) were examined on at least 20 spores using a micrometric eyepiece for each morphotype, except some spores observed in low number. Spore morphotypes were compared with original diagnoses of AMF species and with the reference culture descriptions at http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm and http://zor.zut.edu.pl/Glomeromycota_2/Taxonomy.html. Since important changes of AMF nomenclature have been proposed (Krüger et al. 2009, 2012), with some taxa differently named, we utilised the new binomials only for consistent species names and maintained the previous ones for others.

Total spore densities were determined as spore number 100 g^{-1} soil. Species occurring only in one sample and with a spore number lower than 1% of total spores were excluded from the analyses.

2.4 DNA Extraction from Spores

Intact healthy spores separated in different morphotype were manually collected with a capillary pipette under the dissecting microscope and cleaned by sonication (120 s) in the B–1210 cleaner. After three rinses in sterile distilled water, spores were surface sterilised with 2% chloramine T supplemented with streptomycin (400 µg mL^{-1}) for 20 min and rinsed five times in sterile distilled water. Intact single spores were selected under the dissecting microscope and transferred in Eppendorf tubes before DNA extraction. Spores were individually crushed into 0.2 mL tubes using a glass pestle immediately, and then the DNA extraction was performed with MasterPure yeast DNA purification kit (Epicentre, Madison, USA) according to manufacturer's instructions.

2.5 PCR Conditions of Full-Length SSU-ITS-LSU Sequences

DNA extracts from single spores were used to analyse the ribosomal region comprising the SSU-ITS-LSU fragment using a nested PCR protocol (Krüger et al. 2009). In the first PCR reaction, DNA extracts (1 µL) were amplified in 25 µL of PCR reaction mix using 0.125 U of GoTaq Flexi DNA Polymerase (Promega, Milan, Italy), 0.4 µM of each primer (Af1, Af2 and Ar1, Ar2, Ar3) (Krüger et al. 2009), 0.2 mM (each) dNTPs, 1.5 mM $MgCl_2$ and manufacturer's reaction buffer. The thermal cycler was programmed as follows: initial denaturation at 95 °C for 3 min, 35 cycles at 95 °C for 30 s, 60 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10 min. The nested PCR reactions were performed by diluting (1:100) the first PCR amplicons and using 2 µL of dilutions as template for the second reaction in a final volume of 50 µL. Each primer pair (0.4 µM), SSUmCf1, SSUmCf2, SSUmCf3 and LSUmBr1, LSUmBr2, LSUmBr3, LSUmBr4 (Krüger et al. 2009), were added to the PCR mix. Taq DNA polymerase, dNTPs, buffer and $MgCl_2$ concentrations were as described above. Amplification conditions: initial denaturation at 95 °C for 3 min, 35 cycles at 95 °C for 30 s, 63 °C for 45 s, 72 °C for 1.5 min and a final extension at 72 °C for 10 min. PCR products (10 µL) were separated on 0.8% agarose gels containing ethidium bromide (0.5 µg mL^{-1}).

2.6 Cloning and Sequencing

Amplified DNA fragments of SSU–ITS–LSU regions were purified by Wizard SV Gel and PCR Clean–Up System according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA), with a final elution volume of 20 µL and purified products (2 µL) were quantified by a BioPhotometer (Eppendorf, Hamburg, Germany). Purified products were cloned into pGem-T Easy vector according to the manufacturer's instructions (Promega, Madison, Wisconsin, USA). Positive clones were screened by standard SP6/T7 amplifications, followed by a nested PCR using primer pairs described by Krüger et al. (2009). Concentration of PCR mix components and PCR conditions were the same described above. Amplification products (fifteen clones from each sample) were tested for restriction fragment length polymorphism patterns by digesting the PCR products with HinfI/MboI restriction enzymes (Takara, Madison, Wisconsin, USA), to recover sequence variability. Digested DNA was electrophoresed through 2% MetaPhor agarose (BMA, Rockland, ME USA) containing ethidium bromide (0.5 µg mL^{-1}) and 1 kb Plus DNA Ladder (Invitrogen, Milano, Italy) was used as molecular mass markers. DNA profiles were analysed by Uvitec Cambridge, Essential v4 system. Fifteen clones from single spores (five clones/spore) containing recombinant plasmids with different HinfI patterns were purified by Wizard Plus SV Minipreps (Promega, Madisonm Wisconsin, USA). Recombinant plasmids were sequenced forward and reverse at GATC Biotech (AG European Custom Sequencing Centre, Cologne, Germany). Sequences were edited in MEGAX, and their similarities were determined using the Basic Local Alignment Search Tool (BLASTn) provided by NCBI. The detection of chimeric sequences was performed using USEARCH 6.0 (http://fungen.cme.msu.edu/FunGenePipeline/chimera_check/form.spr). Sequences were aligned with those corresponding to the closest matches from GenBank as well as with sequences from major clades of Glomeromycota using MUSCLE as implemented in MEGAX. Phylogenetic trees were inferred by Neighbor joining analysis. The evolutionary distances were computed using the Maximum Composite Likelihood method. The confidence of branching was assessed using 1,000 bootstrap resamplings. The sequences were deposited in the GenBank database with accession numbers PP422373- PP422391.

2.7 Statistical Analyses

Root colonisation data were analysed by a one-tailed Student t-test. Comparison of colonisation in vineyards with organic and conventional management were analysed independently in every valley. In addition, to assess the combined effects of season and management practices on root colonization across all valleys, we employed a two-way ANOVA for data analysis. Before the analyses, data on root colonisation were arcsine–transformed to satisfy homogeneity of variance to performed ANOVA. Tukey's HSD post hoc comparison was done to test for pairwise mean differences at $p = 0.05$. All analyses were performed using R v4.3.0.

3 Results

3.1 Soil Properties and Grapevine Root Colonisation

Chemical properties of the vineyard soils from Elqui, Casablanca, Cachapoal, Maule, and Itata valleys are shown in Table 1. Differences in N and P content were observed, with higher N and P concentration, and organic matter content in organic/biodynamic vineyards compared with conventional fields. The microscopic examination of the stained grapevine root segments revealed the presence of AMF hyphae, arbuscules and vesicles in the vineyards

Table 1 Description and chemical properties of the sampled sites from Chilean Mediterranean-type ecosystems

Site	Valley (Region)	Location (coordinates)	Agricultural practices	Organic matter (%)	pH	N (mg/kg)	P (mg/kg)
1	Elqui (Coquimbo)	30°4´38.72" S 70° 29´49.79" W	Organic	3.3	7.7	39.6	50.0
2	Elqui (Coquimbo)	30°2´19.72" S 70°41´30.51" W	Conventional	3.2	8.1	13.7	12.8
3	Casablanca (Valparaíso)	33°19´43.93" S 71°26´58.95" W	Organic	2.4	7.4	26.0	54.0
4	Casablanca (Valparaíso)	33°19´30.82" S 71°26´26.19" W	Conventional	1.8	8.2	6.2	8.3
5	Cachapoal (O'Higgins)	34°23´23.17" S 70°47´30.63" W	Biodynamic	7.6	6.5	35.6	68.4
6	Cachapoal (O'Higgins)	34°24´19.26" S 70°50´5.12" W	Conventional	9.0	6.3	20.2	65.0
7	Talca (Maule)	35°58´36.32" S 72°19´23.25" W	Organic	10.0	7.4	61.8	197.0
8	Talca (Maule)	35°58´36.32" S 72°19´23.25" W	Conventional	1.9	5.1	11.3	10.1
9	Itata (Ñuble)	36°46′49.0" S 72°12′58.0" W	Organic	5.9	6.0	24.1	23.0
10	Itata (Ñuble)	36°43′5.13" S 72°21′21.92" W	Conventional	4.5	6.6	7.7	34.9

of the five valleys. The results indicated that the AMF symbioses were present and occurred ubiquitously in Northern, Central and Southern Chile (Fig. 2).

AMF colonisation of grapevine roots ranged from 6 to 35%, showing higher levels in organic/biodynamic management vineyards (20–35%) compared to vineyards with conventional practices (6–31%) (Fig. 3). However, most of the vineyards mycorrhizal colonisation was not affected during different seasons, summer, and winter (Table 2).

3.2 Isolation and Characterisation of AMF Spores in Soils

AMF spores were isolated from the vineyard soils and characterised. Isolated spores were counted (Fig. 4). AMF spores were identified by morphological and molecular analyses (Fig. 5 and 6). The results indicated that Chilean grapevine soils were characterized by a wide variety of AMF. Several morphotypes were identified and described according to characteristics such as spore colour, shape, size, wall type and subtending hyphae (Table 3). In some cases, the taxonomic information was not enough for the accurate characterisation of the species, which was

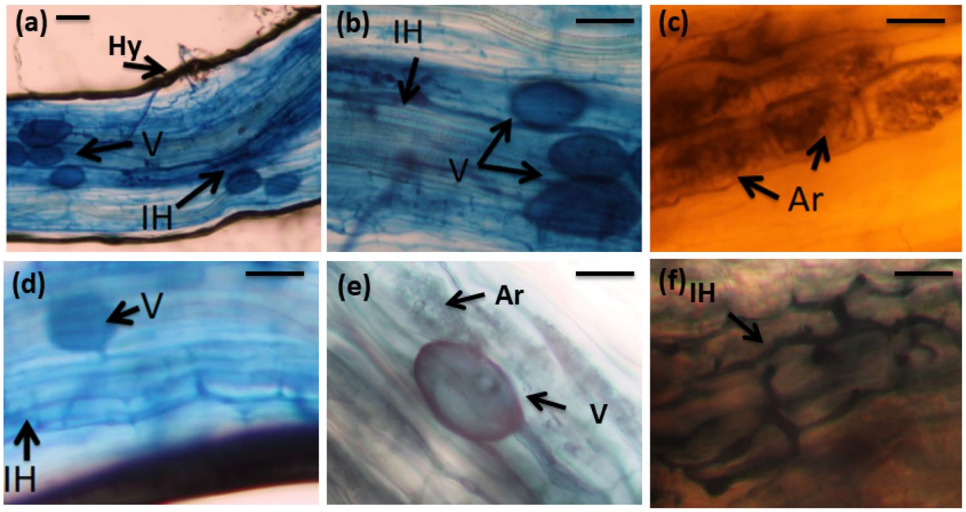

Fig. 2 Typical structures of arbuscular mycorrhizal fungi in Chilean grapevine. Light micrographs showing colonisation pattern in cortex of grapevine (*Vitis vinifera* L.) roots by arbuscular mycorrhizal fungi. (**a**), (**d**) dense mycorrhizal colonisation, showing intercellular hyphae running along the longitudinal root axis and forming many arbuscules and vesicles; (**b**) detail of arbuscules formed within adjacent root cells, showing dichotomous branching of hyphae; (**c**) Dense patches of arbuscules in contiguous cortical root cells of grapevines; (**e**) sparse root colonisation, with rare arbuscules and vesicle; (**f**) intercellular hyphae running along the longitudinal root axis. Bar = 100 µm. Arbuscules (Ar), vesicles (V), appressorium (Ap) and intercellular hyphae (IH) are indicated

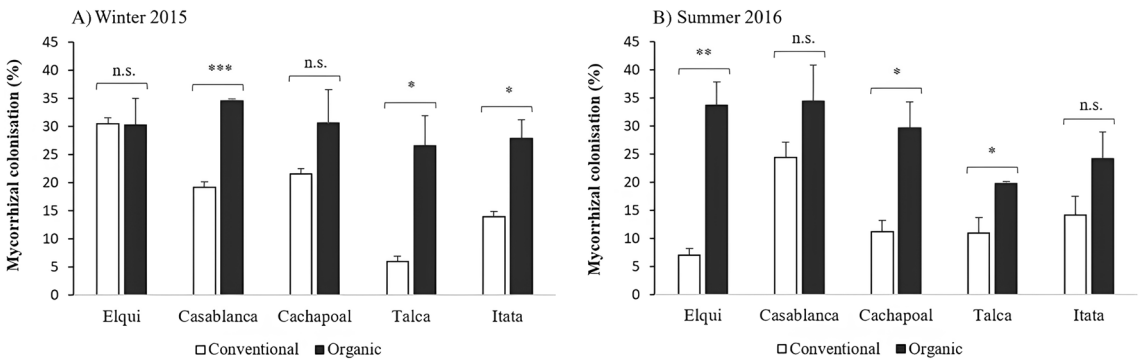

Fig. 3 Mycorrhizal colonisation of grapevine roots in conventional and organic vineyards from Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys. (**a**) Colonisation of grapevines with conventional and organic management in winter 2014; (**b**) Colonisation of grapevines with conventional and organic management in summer 2015. Asterisks indicate significant differences at $p \leq 0.05 = *$, $p \leq 0.01 = **$, $p \leq 0.001 = ***$, according to independent t-test n.s. = not significant.

Table 2 Effects of season and management on mycorrhizal colonisation in grapevine roots

Valley	Winter		Summer		Two-way ANOVA		
	Organic	Conventional	Organic	Conventional	*p* (*s*)	*p* (*m*)	*p* (*s***m*)
Elqui	30.3 ± 5.8	30.6 ± 4.3	33.7 ± 8.1	20.0 ± 3.2	n.s	n.s	n.s
Casablanca	34.6 ± 3.4	22.2 ± 2.7	34.4 ± 3.5	24.4 ± 2.8	n.s	*	n.s
Cachapoal	30.6 ± 4.0	21.6 ± 2.1	29.6 ± 5.7	14.2 ± 2.0	n.s	*	n.s
Maule	26.6 ± 5.4	6.0 ± 1.9	19.8 ± 0.4	10.9 ± 1.8	n.s	**	n.s
Itata	28.0 ± 3.2	14.0 ± 2.1	24.1 ± 2.8	14.2 ± 1.3	n.s	**	n.s

Data are expressed as mean ± standard deviation. p = n.s. (not significant), *(significant at $p \leq 0.05$), or **(significant at $p \leq 0.01$). *s* = season; *m* = management; (*s* × *m*) = season × management

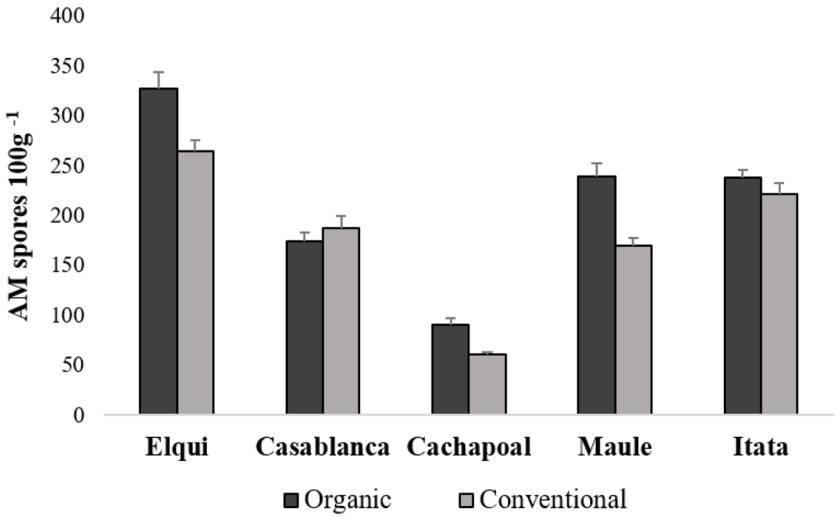

Fig. 4 Soil arbuscular mycorrhizal spores in vineyards from Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys. Soil arbuscular mycorrhiza spores (100 g^{-1}) were measured in conventional and organic management vineyard soils from Elqui, Casablanca, Cachapoal, Maule and Itata Valleys. Data are expressed as mean ± standard deviation

complemented with molecular analyses (Table 4). In accordance with the hypothesis of this study, in organic vineyards higher AMF diversity was observed (Table 5). Some mycorrhizal morphotypes such as *Cetrospora gilmorei* (GI3), *Funneliformis verruculosum* (GL1), *Septoglomus* sp (GL3), *Septoglomus* sp. (GL4) and *Septoglomus constrictum* (GL5) were observed in most vineyards, independent of the agricultural practices. *Funneliformis verruculosum* (GL1)*, Septoglomus constrictum* (GL5), and *Septoglomus* sp. (GL4) were the three most abundant spore morphotypes in vineyard soils (Table 5). In contrast, other morphotypes such as *Acaulospora* sp (AC1), *Scutellospora* sp. (GI1), *Claroideoglomus etunicatum* (GI2), *Septoglomus* sp. (GL2), *Pacispora scintillans* (PA1), *Paraglomus* sp. (PAR1), and *Rhizoglomus sp. (*RG1) showed specific geographical distribution (Table 5). Only in the Southern Maule and Itata Valleys, *Acaulospora* (AC1), *Scutellospora* sp. (GI1), *Claroideoglomus etunicatum* (GI2), *Pacispora scintillans* (PA1) and *Paraglomus* sp. (PAR1) were detected. *Pacispora scintillans* and *Paraglomus* sp. were exclusively observed in organic vineyards. In the Northern Elqui Valley, and Center Casablanca and Cachapoal Valleys, *Septoglomus* sp. (GL2) and *Septoglomus* sp. (GL3) were observed.

Molecular sequence analyses allowed the identification of AMF species belonging to *Septoglomus, Acaulospora, Pacispora* and *Cetraspora* genera in the vineyards from Elqui, Casablanca, Cachapoal, Maule, and Itata valleys (Fig. 6).

4 Discussion

This study is the first large-scale analysis of AMF diversity in Chilean vineyards. This report showed that AM colonisation of grapevine plants highly differed among the vineyards from Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys. In accordance with the proposed hypothesis, the organic/biodynamic practices favoured the establishment of mycorrhizal symbioses in grapevine roots (20 – 35%), while vineyards with conventional management reached lower mycorrhizal colonisation (6 – 31%). Organic/biodynamic vineyards showed generally higher nitrogen, available phosphorus, and organic matter content than conventional vineyards (Table 1). The AMF colonisation levels observed in Chilean vineyards were lower (< 40%) than those reported in vineyards from other geographical locations (Likar et al. 2013; Massa et al. 2020; Schreiner 2020). However, low

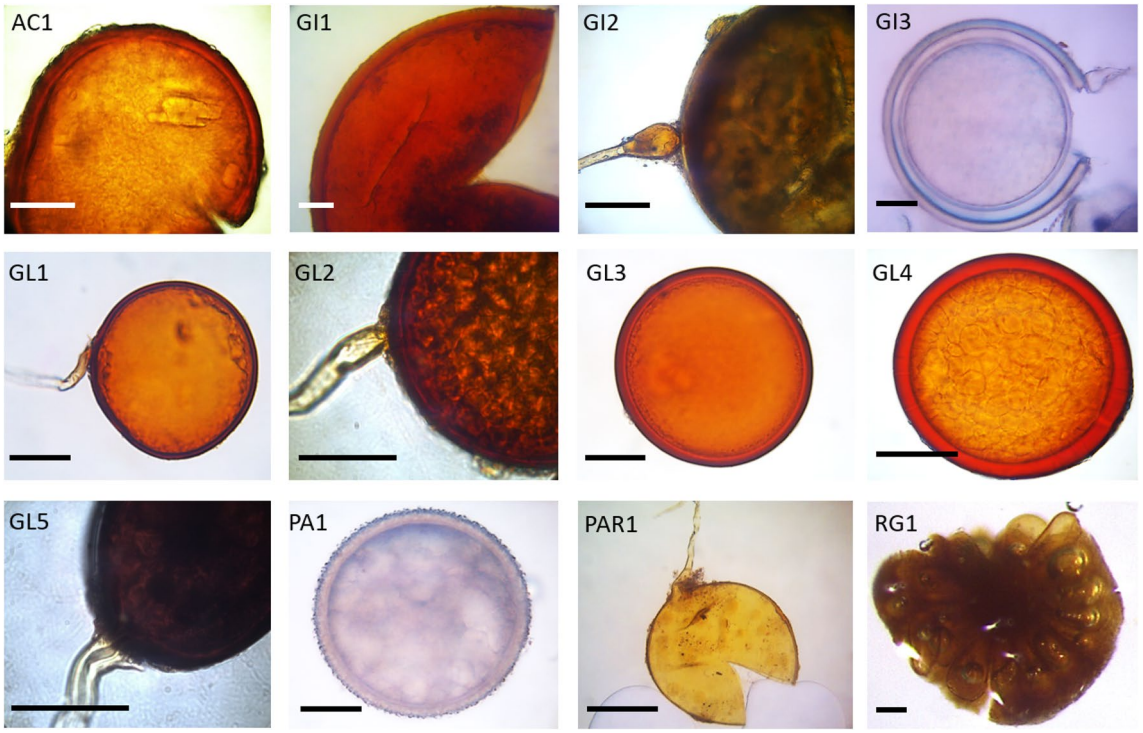

Fig. 5 Grapevine spore morphotypes isolated from vineyard soils from Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys. AC1, *Acaulospora* sp.; PA1, *Pacispora scintillans;* PAR1, *Paraglomus* sp. (*); GI1, *Scutellospora* sp. (*); GI2, *Claroideoglomus etunicatum* (*); GI3, *Cetrospora gilmorei*; GL1, *Funneliformis verruculosum* (*); GL2, *Septoglomus* sp.; GL3, *Septoglomus* sp.; GL4, *Septoglomus* sp.; GL5, *Septoglomus constrictum*; RG1, *Rhizoglomus*sp. (*). Species were classified based on morphological and molecular properties; marked species (*) were classified based solely on morphological properties. Acronyms consider generally two letters of the genus and the number the order of description

levels of mycorrhizal colonisation in grapevines have also been observed in field and in grapevines grown in pots (Camprubí et al. 2008; Schreiner 2007; Velásquez et al. 2020b). Diverse factors may influence the colonisation rates in vineyards, such as mycorrhizal species and strains, geographical conditions, soil properties, cover crops, and agricultural practices (Avio et al. 2006; Munkvold et al. 2004; Schreiner 2020; Trouvelot et al. 2015).

In this study, higher diversity of AMF in Chilean organic vineyards than in conventional fields was observed, which is in accordance with the hypothesis of this study. The analysis of AMF diversity showed high number of *Glomeraceae* spores in vineyards with both organic/biodynamic and conventional managements. Diverse studies on AMF species richness based on DNA amplification of soil or root spores, reported the predominance of glomoid morphotypes and phylotypes (Schreiner and Mihara 2009; Trouvelot et al. 2015). Additionally, a dominance of *Glomeraceae* in the soil and in colonised roots from other ecosystems has been reported (Palla et al. 2020; Trouvelot et al. 2015; Turrini et al. 2017a). Our study showed that specific morphotypes such as *Funneliformis verruculosum* (GL1) and *Cetrospora gilmorei* (GI3) were distributed through all the latitude gradient, whereas other morphotypes were only observed in a specific geographical area. *Septoglomus* sp. (GL4 and GL5) were observed from the Northern Elqui Valley to the Southern Maule Valley but not in the Itata Valley. *Acaulospora* sp. (AC1), *Scutellospora* sp. (GI1), *Claroideoglomus etunicatum* (GI2) and *Pacispora scintillans* (PA1) were observed in the Southern Maule and Itata Valleys, whereas *Paraglomus* sp. (PAR1) was found in Itata Valley. Interestingly, *Pacispora scintillans* (PA1) and *Paraglomus* sp. (PAR1) were observed only in organic vineyards. *Septoglomus* sp. (GL2 and GL3) were present in the Northern Elqui Valley, and Center Casablanca and Cachapoal Valleys. AMF diversity is affected by soil type and land use intensity; some AMF species may be characteristic of a specific area (Oehl et al. 2010). The presence of diverse mycorrhizal genera in Chilean Mediterranean regions, including *Glomus*, *Entrophospora*, *Diversispora, Claroideoglomus, Cetraspora, Archaeospora, Acaulospora, Paraglomus, Pacispora, Gigaspora, Funneliformis, Septoglomus, Scutellospora,* and *Rhizoglomus* has been reported; most AMF belong to the *Glomeraceae* family (Cofré et al. 2019). *Glomus* genus spores were the most abundant in Chilean Matorral and Espinal soils, two typical forests in the Mediterranean climate region

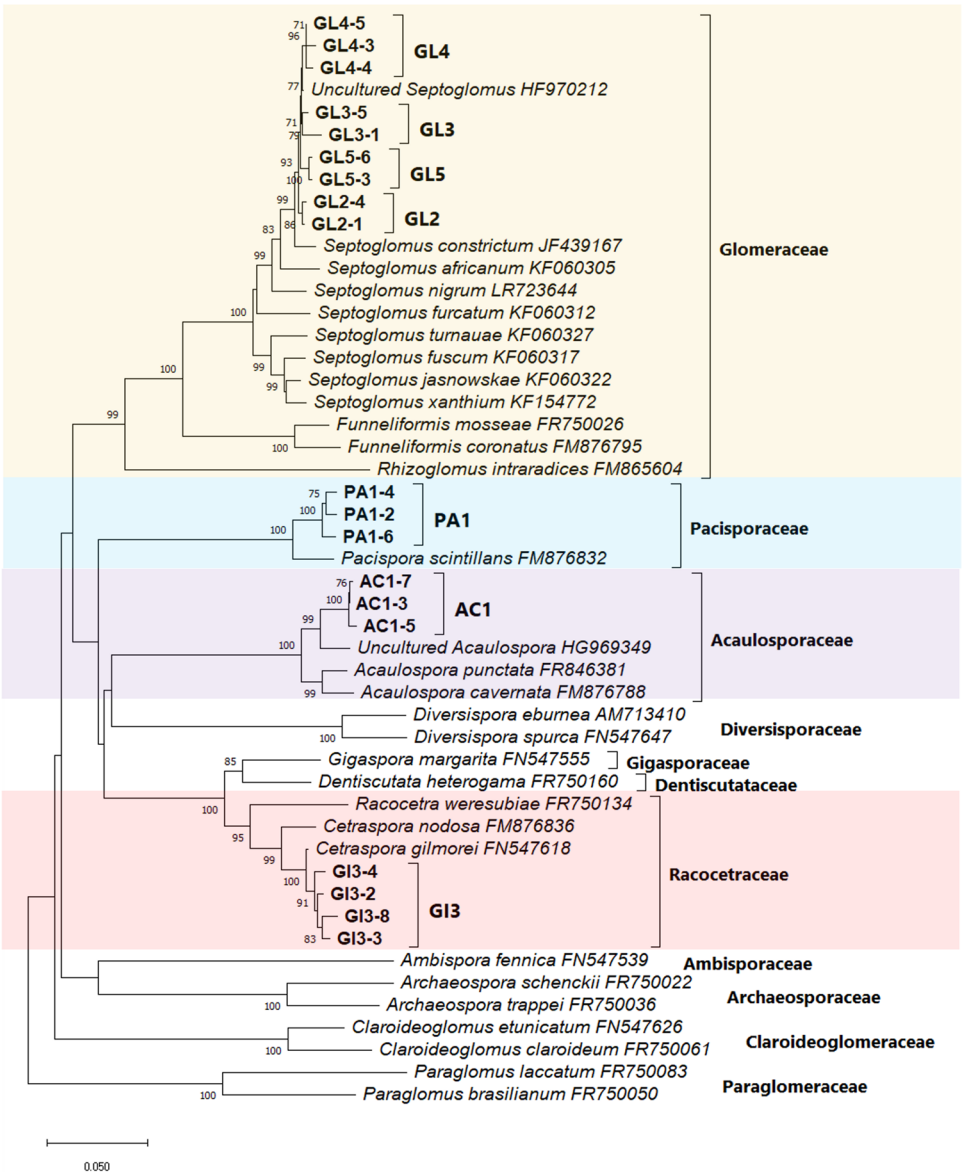

Fig. 6 Phylogenetic tree showing the grapevine arbuscular mycorrhizal fungal species from Elqui, Casablanca, Cachapoal, Maule, and Itata Valleys. Neighbour-joining phylogenetic tree based on the alignment of partial nuclear SSU-ITS-LSU rRNA region (~1500 bp) of different species belonging to *Glomeromycota*. Bootstrap values are shown when they exceed 70% (1000 replications). A sequence of *Paraglomuus brasilianum* was used as outgroup. Sequences obtained in this study are highlighted in bold

(Silva-Flores et al. 2019). However, *Acaulospora* was the most abundant in *Astroceudrus chilensis* tree soils. Our study and other reports indicate that AMF genera and species may have preferences for geographical locations or plant hosts.

In this study, only mycorrhizal spores obtained directly from vineyard soils were analysed, which may differ from species identified in colonised grapevine roots. In vineyards, AMF of the *Glomeraceae* family were reported in mycorrhizal roots, while species of the *Acaulosporaceae* family were not detected in roots but showed abundant spores in the soil (Schreiner and Mihara 2009). These results may be related to the association of AMF species with vineyard green cover/weed plant species other than grapevine plants. Inter-row vegetation in vineyards affects the AMF community, since herbaceous plants play a role in hosting beneficial soil microorganisms (Holland et al. 2014; Jordan et al. 2000). Weeds such as *Plantago lanceolata* and *Tanacetum cinerariifolium* have demonstrated to affect sporulation and development of mycorrhizal intra- and extraradical mycelium in vineyards, increasing the formation of AMF in grapevine. Therefore, green cover plant species may alter AMF diversity in vineyards (Aguilar-Paredes et al. 2020). Compared to conventional vineyards, organic/biodynamic vineyards from the five Chilean valleys here studied showed an increase in mycorrhizal colonisation, spore abundance, and number of species. Similar observations have been reported in previous studies (Oehl and Koch 2018; Turrini et al. 2017a; Verbruggen et al. 2010). Chemical parameters of soils from organic and agroecological vineyards may improve nutritional conditions related to nitrogen, phosphorus, and organic matter

Table 3 Morphological characterisation of arbuscular mycorrhiza fungal spores isolated from vineyard soils

Morphotype	No. of spores analysed	Spore colour	Spore shape	Spore size (µm)	Subtending hypha	Subtending hypha size (µm)	No. of wall layers	Wall layer size (µm)
AC1	100	Yellow	Globose	213 ± 11.08	Glomoid	15.5 ± 0.5	2	15.5 – 6.2
GI1	160	Red – brown	Globose	370 ± 3.53	Gigasporoid	40.2 ± 0.94	2	12.5 – 10.0
GI2	100	Yellow	Globose	240 ± 12.3	Gigasporoid	43.4 ± 0.54	2	15.5 – 6.3
GI3	200	White	Globose	238 ± 1.24	Gigasporoid	46.5 ± 0.23	1	13.0
GL1	108	Yellow – red – brown	Globose	173 ± 8.01	Glomoid	18.6 ± 5.06	2	2.0 – 13.6
GL2	200	Red – brown	Globose	163 ± 3.31	Glomoid	15.5 ± 0.47	2	15.5 – 12.4
GL3	201	Orange	Globose	175 ± 3.55	Glomoid	16.6 ± 0.1	2	6.0 – 12.7
GL4	200	Orange	Globose	160 ± 3.03	Glomoid	15.5 ± 0.37	2	9.3 – 6.2
GL5	200	Yellow – red – brown	Globose	108 ± 6.27	Glomoid	5.6 ± 0.14	3	2.5 – 6.3 – 3.8
PA1	100	White	Subglobose	186 ± 3.6	Glomoid	9.3 ± 0.11	2	1.3 – 1.3
PAR1	200	White – Yellow	Subglobose	116 ± 1.65	Glomoid	4.7 ± 0.09	2	6.3 – 5.0
RG1	200	Orange – Brown	Subglobose	438 ± 2.84	N.O	N.O	N.O	N.O

Morphological characterisation was performed following the taxonomic keys to identify *Glomeromycota* species (http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm and http://www.agro.ar.szczecin.pl/~jblaszkowski/index.html). Data are expressed as mean ± standard error (n = 10)

Table 4 SSU–ITS–LSU rRNA region sequences obtained from arbuscular mycorrhizal fungal spore morphotypes from Chilean vineyards

Sequence name	Accession number	Identity (%)	Taxonomic affiliation
AC1-3	PP422385	HG969349 (97.40)	Uncultured *Acaulospora*
AC1-5	PP422386	HG969349 (96.99)	Uncultured *Acaulospora*
AC1-7	PP422387	HG969349 (97.26)	Uncultured *Acaulospora*
GI3-2	PP422373	FN547618 (98.89)	*Cetraspora gilmorei*
GI3-3	PP422374	FN547610 (98.55)	*Cetraspora gilmorei*
GI3-4	PP422375	FN547618 (99.03)	*Cetraspora gilmorei*
GI3-8	PP422376	FN547618 (98.21)	*Cetraspora gilmorei*
GL2-1	PP422390	HF970212 (98.72)	Uncultured *Septoglomus*
GL2-4	PP422391	HF970212 (98.59)	Uncultured *Septoglomus*
GL3-5	PP422383	MT765663 (98.91)	*Septoglomus* sp.
GL3-1	PP422384	MT765663 (98.21)	*Septoglomus* sp.
GL4-3	PP422380	HF970212 (98.29)	Uncultured *Septoglomus*
GL4-4	PP422381	HF970212 (98.48)	Uncultured *Septoglomus*
GL4-5	PP422382	HF970212 (98.92)	Uncultured *Septoglomus*
GL5-6	PP422388	HF970212 (98.60)	Uncultured *Septoglomus*
GL5-3	PP422389	HF970212 (98.66)	Uncultured *Septoglomus*
PA1-2	PP422377	FM876832 (96.36)	*Pacispora scintillans*
PA1-4	PP422378	FM876832 (96.36)	*Pacispora scintillans*
PA1-6	PP422379	FM876832 (96.29)	*Pacispora scintillans*

content as observed in most vineyards in this study, despite their scarce external fertilisation (Altieri et al. 1996). The agricultural practices of organic systems, including use of mixed permanent coverages, reduction of agricultural chemicals, as well as use of polycultures, enhance the activity and diversity of arbuscular mycorrhizal species in the soil, promoting a higher resilience of the agricultural systems to both biotic and abiotic stresses (Aguilera et al. 2022; Bender et al. 2016).

In addition, root colonisation pathways differ among arbuscular mycorrhizal fungus families. Species in the *Glomerales* order primarily colonise plant roots starting from hyphal fragments, while root colonisation by *Diversisporales* starts from spores, suggesting that *Diversisporales*

Table 5 Distribution (%) of arbuscular mycorrhiza fungal spore morphotypes observed in Chilean vineyards

Morphotype	Vineyards fields									
	Elqui		Casablanca		Cachapoal		Maule		Itata	
	1	2	3	4	5	6	7	8	9	10
Acaulospora sp. AC1							19.2		22.7	
Scutellospora sp. GI1								59.2	25.2	
Claroideoglomus etunicatum GI2								26.0		25.3
Cetrospora gilmorei GI3	23.2		1.7	2.1	23.3	36.7		5.3		29.4
Funneliformis verruculosum GL1	4.9		27.0		23.3		6.3		3.8	
Septoglomus sp. GL2	30.6			53.5						
Septoglomus sp. GL3	13.8	18.2	21.8	21.4	18.9	21.7				
Septoglomus sp. GL4	11.3	28.0	19.0	7.0	17.8	8.3	5.9	4.7		
Septoglomus sp. GL5	8.0	15.9	23.0	16.0	16.7	33.3	7.9	4.7		
Pacispora scintillans PA1							35.6		6.3	
Paraglomus sp. PAR1									42.0	45.2
Rhizoglomus sp. RG1	8.3	37.9	7.5				25.1			
Total (%)	100	100	100	100	100	100	100	100	100	100

fungi are slower colonisers than *Glomerales* (Hart and Reader 2002a, b). Therefore, colonisation rates of grapevine plants may also depend on the composition of the mycorrhizal community.

Concerning to managements, we observed only minor differences in mycorrhizal colonisation in winter and summer (Table 2). It has been reported that mycorrhizal activity and diversity may be seasonally stable but may change depending on vineyard age and soil type (Schreiner and Mihara 2009). In contrast, other reports have described higher mycorrhizal activity in summer compared to winter, probably associated to an increased nutrient absorption related to vegetative growth or seasonal water stress (Schreiner 2005; Sosa-Hernández et al. 2019). Recently, Aguilera et al. (2024) analysed AMF communities in Chilean conventional vineyards, reporting no effects of grapevine cultivar, age, and geographic location on AMF richness.

5 Conclusions

This pioneering large-scale study on the diversity of arbuscular mycorrhizal fungi in ten Chilean vineyards revealed a dominance by the Glomeraceae family, consistent with findings from other vineyard studies. The presence of the species *Glomus, Rhizoglomus, Septoglomus, Funneliformis, Claroideoglomus,* and *Paraglomus* were determined. Generalist species were present in all valleys, while some species showed geographical restrictions. A significant increase in the diversity of arbuscular mycorrhizal fungi was noted in organic and biodynamic vineyards compared to conventional vineyards, probably due to sustainable agricultural practices that enhance mycorrhizal colonization, abundance, and biodiversity. No significant seasonal variation in symbiosis or species succession was observed between summer and winter.

Our findings contribute to understanding the ecology of these beneficial fungi, developing native inoculants to improve the nutraceutical properties of wines. This study provides essential knowledge for future applications in sustainable agricultural practices, such as organic methods that promote the ecosystem services of soil microorganisms. Future long-term studies on soil microorganisms in vineyards should be conducted to further characterise the impact of sustainable agricultural practices.

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Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by AA, AT, LA, AV, CS and MS. The first draft of the manuscript was written by AA and MS. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data Availability All data generated or analysed during this study are included in this published article.

Declarations

Ethics Approval The authors declare that this study is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Consent to Participate Informed consent was obtained from all authors for participation.

Consent for Publication The authors have consented to the publication.

Conflicts of Interest/Competing Interests The authors have no conflicts of interest to declare that are relevant to the content of this article.

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