OUTSIDE AND INSIDE GRAPEVINE ROOTS: ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES IN A ‘NEBBIOLO’ VINEYARD [•]

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1. INTRODUCTION

In wine grape production mineral fertilizer and cover crops are combined to optmize the soil nutrient content. A low fertilizer input can be applied, since grapevine roots are colonized by arbuscular mycorrhizal fungi (AMF) under field conditions. The root symbiotic fungi that form arbuscular mycorrhizae (phylum *Glomeromycota*) are among the most important soil organisms. They are the oldest group of organisms living in symbiosis with land plants (Redecker *et al.*, 2000; Bonfante, Genre, 2008). AMF facilitate mineral nutrient uptake from the soil in exchange for plant-assimilated carbon and promote water-stress tolerance and resistance to certain diseases (Smith, Read, 2008). The inoculation of vine rootstock with AMF can result in increased growth (Schubert *et al.*, 1988), enhanced nutrient uptake (Schreiner, 2007) and improved drought tolerance e.g., in ‘Cabernet sauvignon’ grafted onto various rootstocks (Nikolaou *et al*., 2003). Increased root colonization by AMF in response to a lower soil water content implies that AMF may play a significant role in the response of vines to water stress (Schreiner *et al.*, 2007). However, little is known about the species composition of AMF communities associated to grapevine roots in vineyards. Previuos studies, based on the identification of AMF spores, reported the genus *Glomus* as being the most represented in vineyards (Karagiannidis *et al.*, 1997). However, the spores were not able to mirror the AMF community present in the soil due to the seasonality and their different production rate. Recently, the use of a molecular approach has allowed this gap in knowledge to be overcome (Schreiner, Mihara, 2009; Balestrini *et al.*, 2010). Balestrini *et al.* (2010) have carried out an investigation on AMF communities in vineyards by means of the analysis of partial rRNA gene sequences. Two vineyard soils, with different physical-chemical features, were compared and the results have shown that the soil characteristics can play an important role and shape the AMF assemblage structure and composition, thus confirming previous studies (Schreiner, Mihara, 2009; Lumini *et al.,* 2010). The aim of this study is to characterize the AMF community in

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[•] QUAD. VITIC. ENOL. UNIV. TORINO, 31, 2009-2010 association with grapevine roots in a Piedmont vineyard and to analyze the relationship between AMF communities outside and inside roots.

2. MATERIALS AND METHODS

2.1. Site description and sampling

Our study was set up in a cover cropped vineyard located in Neive (44° 43' 16" N; 8° 4' 59" E; 260 m above sea level), in a typical hilly Piedmont landscape (Langhe, Italy). Six plants belonging to *Vitis vinifera* cv 'Nebbiolo' clone 308, grafted onto '420A' rootstock, were randomly chosen for sampling from the vineyard. Three subsurface soil cores (50 mm Ø and 0,20 m depth) and young root fragments were collected from each plant on 15 May 2008, frozen in liquid nitrogen, and stored at -80 °C.

2.2. Molecular analyses

DNA extraction was performed according to Balestrini *et al.* (2010). GAPDH-f and GAPDH-r primers (Reid *et al.*, 2006), designed for *Vitis vinifera* GAPDH, were employed in PCR amplifications as a positive control of root DNA. The species composition of the AMF intraradical community was analyzed using a nested PCR approach directly to amplify a small portion (550 bp) of AM fungal SSU rDNA and the PCR products were cloned and sequenced, as described in Balestrini *et al.* (2010). The sequences were deposited at the National Centre for Biotechnology Information (NCBI) GenBank with accession numbers HQ263038-HQ263108 (available online).

2.3 Alignment, clustering and phylogenetic analyses

The sequence similarities were determined using the BLASTn sequence similarity search tool provided by GenBank. Root sequences were then aligned and grouped in OTUs, as described in Balestrini *et al.* (2010). Consensus sequences for each $OTU_{0.02}$ and reference sequences from GeneBank, representative of the major groups described by Schüβler *et al.* (2001), were employed in a Neighbor-Joining phylogenetic analyses together with sequences previously obtained from soil of the same vineyard (Balestrini *et al.*, 2010).

3. RESULTS AND DISCUSSION

In order to have a better representation of the symbiotic community under study, PCR was performed on two different root fragments from each plant, to compare the data obtained from the roots with those previously obtained from the soil (Balestrini *et al.*, 2010). Nested amplification was performed using the couple of primers NS31-AMmix (AM1, AM2 and AM3), which amplify DNA from a wide range of taxa belonging to *Glomeromycota* (Santos-Gonzales *et al.*, 2007). Forty-eight clones from each plant were screened, and then 16 clones were chosen (in total of 96 sequences) for sequencing. Out of the 96 sequenced clones, 15 were discarded because of poor quality, while 71 (87.6 %) were identified, by means of BLASTn analyses, as belonging to

Glomeromycota taxa and assigned to 16 operational taxonomic units (OTUs), with a 98 % similarity level. The distribution in the several OTUs showed that the 3 most representative OTUs in the roots grouped more than 50 % (54.9 %) of sequences. Phylogenetic analyses highlighted the presence of AMF in the roots belonging to only *Glomus* Group A (*Glomeraceae* family) (fig. 1). The OTU comprising the highest number of sequences (31 %) is positioned in the clade related to *Glomus sinuosum*, which belongs to *Glomus* subgroup Ab.

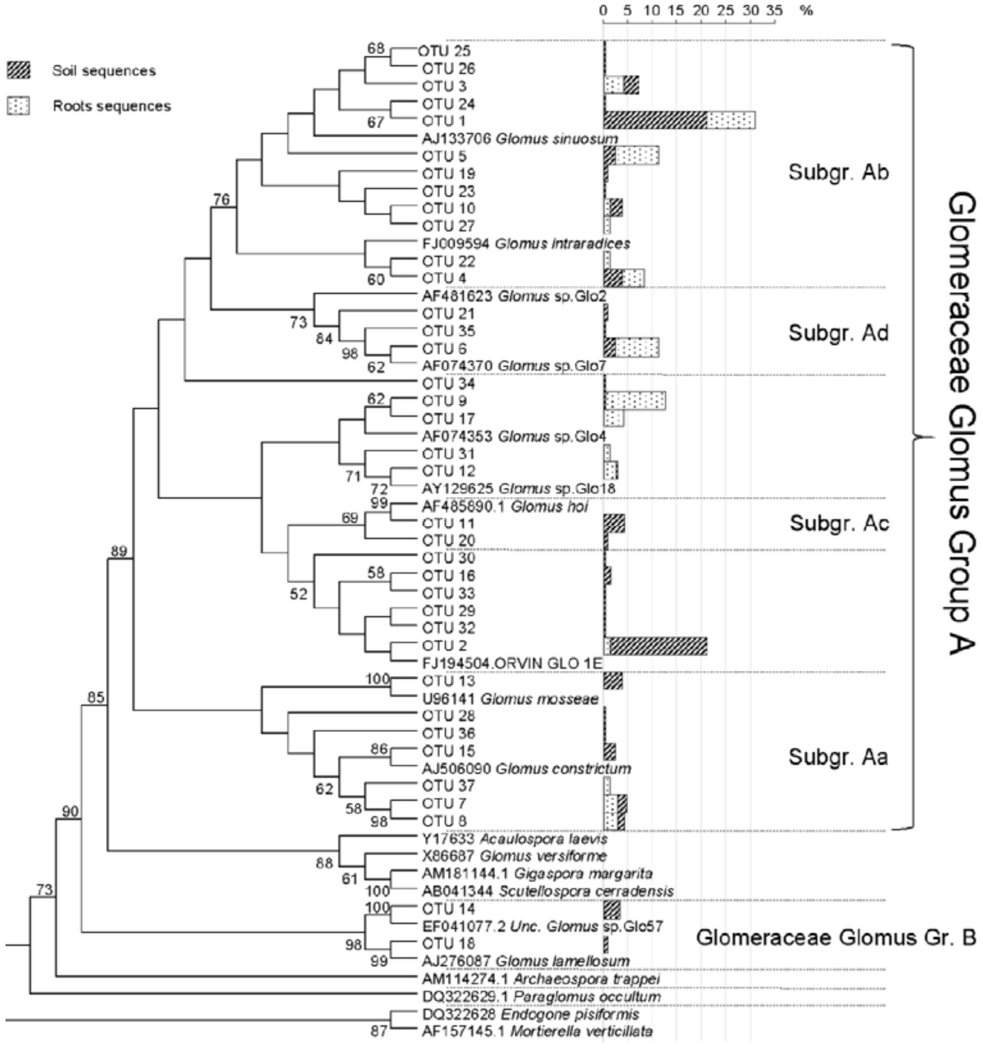

Fig. 1. Neive AMF communities (roots and soil) phylogenetic tree based 011 550 bp of SSU rDNA sequences. Numbers near the branches are bootstrap values from 10000 resamplings determined for NJ analysis. *Endogone piszformis* and *Mortierella verticillata* were used as outgroups. The set of OTUs employed was generated with soil (Balestrini *et al.*, 2010) and roots sequences pooled. In the histogram, each OTU is shown as sequences percentage in soil/root compartments.

Considering previous data from a soil analyses conducted on the same vineyard (Balestrini *et al.*, 2010), it was possible to evaluate the overlap between the root and soil AMF communities. Eleven OTUs were found in common, suggesting a good correlation between the two compartments. Four of the most representative OTUs found in the roots were also present in the soil compartment with a good number of sequences. The highest number of sequences from the soil and roots were found in the same OTU (OTU1). Some OTUs result to be present exclusively in one of the two compartments: *Glomus* Group B and *Glomus* group A subgroup Ac are present in the soil communities, but not in the roots. In addition, the OTU2, related to the phylotype ORVIN GLO 1E (Schreiner, Mihara, 2009) is highly represented in the soil compartment (21.3 %), while it has been found as a singleton (one sequence) in the roots. These results could be explained by the different level of symbiosis competitiveness on the grapevine roots shown by the different phylothypes present in the soil. However, taken together the data obtained from the Neive cover cropped vineyard, cultivated with the *Vitis vinifera* 'Nebbiolo', confirm the good correlation between the soil and root communities reported by Balestrini *et al.* (2010) for another Piedmont cover cropped 'Nebbiolo' vineyard.

In conclusion, the results obtained in this study, together with the few so far published on vineyard ecosystems (Schreiner, Mihara, 2009; Balestrini *et al.*, 2010; Lumini *et al.*, 2010), highlight the AMF phylotypes that are most commonly found in vineyards and those that are more efficient in engaging symbiosis with grapevine roots.

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Abstract

In field conditions, grapevine roots are colonized by arbuscular mycorrhizal fungi (AMF). Little is known about the species composition of AMF communities associated to grapevine. Recently, the use of the molecular approach has offered new information about the AMF assemblages that live in symbiosis with this important typical Mediterranean fruit crop. The aim of this study was to compare the AMF communities outside and inside the grapevine roots in a 'Nebbiolo' Piedmont vineyard, in order to investigate the relationship between the AMF communities in these two compartments.

Literature cited

Balestrini R., Magurno F., Walker C., Lumini E., Bianciotto V. - 2010 - Cohorts of arbuscular mycorrhizal fungi (AMF) in *Vitis vinifera*, a typical Mediterranean fruit crop. *Environmental Microbiology Reports*, 2, 594-604.

Bonfante P., Genre A. - 2008 - Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends in Plant Science*, 13, 492-498.

Karagiannidis N., Velemis D., Stavropoulos N. - 1997 - Root colonization and spore population by VA mycorrhizal fungi in four grapevine rootstocks. *Vitis*, 36, 57–60.

Lumini E., Orgiazzi A., Borriello R., Bonfante P., Bianciotto V. - 2010 - Disclosing arbuscular mycorrhizal fungal biodiversity in soil through a land-use gradient using a pyrosequencing approach. *Environmental Microbiology*, 12, 2165-2179.

Nikolaou N., Angelopoulos K., Karagiannidis N. - 2003 - Effects of drought stress on mycorrhizal and nonmycorrhizal Cabernet Sauvignon grapevine, grafted onto various rootstocks. *Experimental Agriculture*, 39, 241–252.

Redecker D., Kodner R., Graham L.E. - 2000 - Glomalean Fungi from the Ordovician. *Science*, 289, 1920-1921.

Reid K., Olsson N., Schlosser J., Peng F., Lund S. - 2006 - An optimized grapevine RNA isolation procedure and statistical determination of reference genes for realtime RT-PCR during berry development. *BMC Plant Biology*, 6, 27.

Santos-Gonzalez J.C., Finlay R.D., Tehler A. - 2007 - Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Applied Environmental Microbiology*, 73, 5613-5623.

Schreiner R.P. - 2007 - Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of ‘Pinot noir’ (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. *Applied Soil Ecology*, 36, 205-215.

Schreiner R., Tarara J., Smithyman R. - 2007 – Deficit irrigation promotes arbuscular colonization of fine roots by mycorrhizal fungi in grapevines (*Vitis vinifera* L.) in an arid climate. *Mycorrhiza*, 17, 551–562.

Schreiner R.P., Mihara K. - 2009 - The diversity of arbuscular mycorrhizal fungi amplified from grapevine roots (*Vitis vinifera L.*) in Oregon vineyards is seasonally stable and influenced by soil and vine age. *Mycologia*, 101, 599-611.

Schubert A., Cammarata S., Eynard I. - 1988 - Growth and root colonization of grapevines inoculated with different mycorrhizal endophytes. *Horticultural Science*, 23, 302-303.

Schüβler A., Schwarzott D., Walker C. - 2001 - A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research*, 105, 1413-1421.

Smith S.E., Read D.J. - 2008 - *Mycorrhizal symbiosis*. Ed. Academic Press, Amsterdam, NL.