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Mycorrhizal Fungi Collected from the Rhizospheres around Different Olive Cultivars Vary in Their Ability to Improve Growth and Polyphenol Levels in Leeks

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Abstract

Mycorrhizal fungus spores and propagules were collected from the soils in the vicinity of roots of five different olive cultivars. These mycorrhizal fungus communities were amplified in trap cultures and then their effect on the growth and polyphenol levels of leek plants was determined. All mycorrhizal fungus communities increased plant growth in leeks when compared to controls. In addition, communities from the roots of Frantoio and Manzanillo significantly increased plant growth, in terms of plant height and dry weights, as compared to plants that were given mycorrhizal fungus collected from cultivar Mission. Plants inoculated with mycorrhizal fungus from Frantoio also had an increase in 14 polyphenols compared to uninoculated plants. A majority of polyphenol peaks were also higher in leek plants inoculated with mycorrhizal fungi from Frantoio roots when compared to plants inoculated with mycorrhizal fungi from other olive cultivars. The affected polyphenols were identified by mass spectrometry and were mostly found to be derivatives (e.g., pentose, hexose, malonyl, feruyl, and coumaroyl) of quercetin, kaempferol, and apigenin; four remained unidentified. Molecular fingerprinting of mycorrhizal fungus communities from different olive cultivars indicated that fungi of the family Gigasporaceae were a major component of inocula obtained from Frantoio and Manzanillo roots, which were better performers in terms of plant growth and polyphenol content. Mycorrhizal fungi from cv Mission roots were relatively poor performers and were dominated by the mycorrhizae of the family Glomeraceae, specicifally the genus *Rhizophagus*.

Keywords: roots, polyphenols, plant growth, mycorrhizal fungi

1. Introduction

Over two hundred arbuscular mycorrhizal [AM] fungus species have been identified and many more may be identified in days to come (Wang et al., 2011). In general, AM fungi are ubiquitous in their symbiotic relationship with various terrestrial plant species (Smith & Reed, 1997). However, considering that a vast variety of plant cultivars and a huge number of AM fungi species exist in the same ecosystem, it is plausible that certain combinations of specific plant cultivars and specific AM fungi species will develop preferential symbiosis that would lead to better plant growth and production. In this context, it is noteworthy that some AM fungi species do exhibit host specificity (Cho et al., 2006). On the other hand, it is also likely that several mycorrhizal species may colonize the same root or different roots of a plant within their micro-ecosystem (Chilvers et al., 1987; Sanders et al., 1977). Ideally, our goal is to find combinations of specific AM fungi species and plant cultivars that perform best for plant growth and quality, and hence offer opportunities to improve production in our cultivation systems. Therefore, our study was conducted to isolate communities of AM fungi from the rhizospheres of different olive cultivars to study which of these perform best in terms of improving plant growth and/or polyphenols of the host plant, under a controlled experimental system, which could later be exploited for olive field studies.

Phenolic compounds are widely distributed among plants and over 5000 species of phenolic compounds have been noted in various plants (Shahidi & Naczk, 1995). These secondary metabolites are well known for two

major roles: i) health benefitting compounds, and also, ii) plant defense chemicals. Health benefits of different types of phenolic compounds include, but are not limited to, protection against atherosclerosis, hypertension, cardiovascular diseases, cancer, viral and bacterial infections (Owen et al., 2000; Choi et al., 2002; Fraga et al., 2010). The role of polyphenols in plant defenses has been noted by a number of researchers (Feeny, 1976; Jones & Klocke, 1987; Lattanzio et al., 2006). Any agent, such as AM fungus, that increases polyphenols should be of interest to agricultural researchers and farmers, and therefore, determination of changes in the levels of polyphenols in test plants inoculated with AM fungi communities isolated from different olive cultivars was made an important part of this study.

Olive cultivars grown in Texas were chosen for this study because olives are being introduced as a promising cash crop in Texas (Malik & Bradford, 2004; Malik & Bradford, 2005a; Malik & Perez, 2010; Selin et al., 2012). It is still a new crop and therefore its cultivation could benefit substantially if appropriate mycorrhizal fungi inocula were introduced along with the appropriate cultivar for optimal yield and quality. Our previous studies had shown that mycorrhizal fungi could increase polyphenol contents in different host plants (Malik et al., 2015; Malik et al., 2016). Since olives are well recognized for their valuable polyphenols (Amiot et al., 1986; Manna et al., 2002; Malik & Bradford, 2008), a good olive AM fungal combination could be highly beneficial for olive cultivation.

2. Material and Methods

2.1 Olive Root Soil Samples

Five olive (*Olea europaea* L.) cultivars, grown at the Sandy Oaks farm at Elmandorf, Texas (about 20 miles from San Antonio, TX), were chosen for this study, among them 'Arbequina', which was included because it is the most recommended cultivar for Texas, and 'Frantoio', which was included because it is generally perceived that its roots are more resistant to soil-borne pathogens (Bibici & Cirulli, 2012). The other cultivars were 'Manzanillo', 'Koroneiki', and 'Mission'. The plants chosen were at least eight years old. Three replicate trees of each cultivar were randomly selected for collection of soil samples. Multiple soil samples were taken from around roots of each replicate tree of a cultivar and were combined as one composite sample before shipping to USDA-ARS research center in Wyndmoor, PA where they were stored at 4°C until further processing.

2.2 Multiplication of Mycorrhizal Fungi in Trap Cultures

The AM fungi in the soil samples were multiplied in trap cultures by the following method. A 65 ml plastic cone was used for trap cultures as described earlier (Walker, 1999). The bottom of the cone was plugged with polyester fiber and then the bottom 1/3 portion was filled with our growth mix: SSVT (sand: soil: vermiculite: turface (2:1:2:1), which had been autoclaved. On top of the SSVT, the rhizosphere soil sample from olive cultivars (test soil sample: TSS) was poured to another 1/3 volume of the cone. On top of the TSS layer, SSVT was poured to fill the remaining 1/3 volume of the cone. The cone was immediately watered with Hoagland solution without phosphorus (Hoagland & Arnon, 1939), and received a two month old seedling of Bahiagrass (*Paspalum notatum* Flugge). Plants were grown for five months in a growth chamber, maintained at a 14 h photoperiod (18 °C in the night and 25 °C during day) and during this time they received weekly fertilization without phosphorus.

2.3 Plant Growth and Polyphenol Analyses of Leeks

2.3.1 Plant Growth Tests on Leeks

TSS, plus SSVT, from six trap culture cones, representing a single specific olive cultivar, were pooled together and thoroughly mixed. Using this mix (TRP) in place of TSS, twelve new cones were prepared as described above for trap cultures. This time, instead of planting Bahiagrass, one-month old seedlings of leek (*Allium porum* L. cv Lancelot) were planted in the cones and the cones were supplied with Hoagland solution, containing $1/10^{th}$ strength of phosphorus, every five days. This setup was prepared for each of the olive cultivar rhizosphere/trap culture soil samples. Side by side with these cones, twelve non-mycorrhizal control cones were also prepared, where autoclaved trap culture soil was used along with autoclaved SSVT. The leeks in these cones were grown for eight weeks under the same conditions as described above, then removed from the cones, and washed to measure shoot length and fresh weight. The roots were stained (Phillips & Hayman, 1970) to check colonization of roots by AM fungi via the gridline intersect method (Giovannetti & Mosse, 1980). These analyses determined that the inoculated plants were heavily colonized while control plants were clean, thus, confirming the suitability of these plants for this study. Six leek shoots harvested from each group were reduced to three pooled samples of two leeks. These samples were frozen at -80 °C until extraction and analysis for polyphenols as described below. The remaining six plants, from each group, were used to determine dry weight (80 °C) and for mineral analysis.

2.3.2 Polyphenol Extraction

The frozen plant material was pulverized in liquid nitrogen and 200 mg aliquots of this powder were extracted in 80% methanol to a final volume of 2 mL, as described earlier (Malik & Bradford, 2005b). The extracts were stored at -80 °C until analyzed. Just before analysis by HPLC-MS, a 200 µL aliquot was taken from the extract and reduced under vacuum to 50 µL.

2.3.3 Chromatographic Analysis

The chromatographic separation of the extract was performed with a Nano-Acquity (Waters, Milford, MA) ultra-high performance liquid chromatographer (UHPLC), equipped with an Acquity UPLC BEH C18, 1.7 µm (1 × 100 mm) column (Waters) and maintained at 40 °C and running at 60 µl/minute. The UHPLC-UV chromatogram was obtained at 280 nm with an Acquity TUV (Waters) as the detector. The method used for analysis and sample preparation was as previously reported by Malik et al. (2015). The UHPLC gradient started with water-acetonitrile 95:5 (0.1% formic acid) for 2 minutes and ramped linearly to water-acetonitrile 60:40 (0.1% formic acid) at a final time of 14 minutes, maintained at that solvent composition for 2 minutes and followed with a column wash of water-acetonitrile 20:80 (0.1%) formic acid) and returning to the initial condition at 20 minutes, allowing 10 minutes for stabilization. A 10 µl of kaempferol solution (internal standard, 5 µg/ml) was added to each sample. The solvent was removed under nitrogen, followed by re-suspension of the sample in 50 µl of water-methanol 90:10. Three injections of 4 µl were made for each sample.

2.3.4 Mass Spectrometry Analysis

The mass spectrometry analysis was accomplished by connecting the effluent of the UHPLC instrument to a Synapt G1 quadrupole-time of flight mass spectrometer (Waters) operating in the W mode (resolving power of 18,000) and with an electrospray ionization (ESI) probe, operated in the positive mode and controlled by MassLynx v.4.1 software (Waters). The instrument parameters and calibration procedures were as previously reported (Malik et al., 2015). The separation of polyphenols in the extract and identification of specific peaks was performed using the same instruments and methodology as described previously (Malik et al., 2015). The only difference was that the extracts were concentrated at least four fold; and therefore, larger number of peaks were observed, which were quantified and identified using the same methods as previously described (Malik et al., 2015).

2.4 Identification of Spores by Molecular and Morphological Methods

2.4.1 Spore Isolation

Spores of the AM fungi were isolated from the soil via wet sieving and centrifugation (Gerdemann & Nicholson, 1963; Jenkins, 1964).

2.4.2 DNA Extraction from Single Spores

The DNA from single spores was isolated following the method of Jansa et al., 2002. Briefly, spores were individually pipetted into a 2 ml Eppendorf tube for sterilization with Streptomycin, Gentamicin and Chloramine T, as described by Horii & Ishii, 2006. Sterile spores were pipetted into a PCR tube (50 µl, thin-walled) and DNA was extracted by crushing the spore with a pipet tip, followed by heating for 1 minute at 95 °C in the presence of 10 µl 20% w/v Chelex-100 Resin (Bio-Rad). The DNA extract was cooled on ice for 5 minutes, and then 5 µl of DNA extract was set aside for PCR amplification of a specific region of DNA as described below.

2.4.3 Amplification of DNA by PCR Followed by Confirmation and Purification

A portion of the SSU rRNA gene was amplified using nested PCR following the method of Lee et al. 2008. The first round of PCR was conducted using NS1 and NS4, which are universal eukaryotic primers (Van-Tuinen et al., 1998). The PCR reaction was performed using 45 µl of Platinum® PCR SuperMix (Invitrogen, which containd 20 U/ml Taq DNA polymerase with Platninum® Taq Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 µm dNTP). To the SuperMix, 1 µl of each of the primers NS1 and NS4 (Integrated DNA Technologies) were added, giving a 250 nM concentration of each primer in the final mix. The volume of the reaction mix was adjusted to 48 µl with DEPC-Treated Water (Ambion). Finally, 2 µl of DNA isolate was added to the reaction, bringing the final volume to 50 µl. The PCR reaction was performed as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 42 °C for 1 min, and extension at 72 °C for 1 min, and followed by a final extension at 72 °C for 10 min. PCR products were diluted 1:50 with DEPC-Treated Water, before nested round of PCR. The nested round of PCR was performed using primers AML1 and AML2 (Lee et al., 2008), with the same reaction mix conditions as the first round of PCR, using the diluted PCR product from the first round. The PCR reaction was conducted as follows:

initial denature at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, anneal at 50 °C for 1 min, and extension at 72 °C for 1 min, and followed by final extension at 72 °C for 10 min.

To confirm a positive amplification of DNA, PCR products from the nested round were electrophoresed on a 1.5% agarose gel at 80 V for 30 minutes. The agarose gel was prepared by heating 1.5 g of certified molecular biology agarose (Bio-Rad) in 100 ml 1%TAE (Boston BioProducts), followed by cooling to 55 °C and adding 9 µl SYBR SAFE Stain (Invitrogen). Finally, the gel medium was poured on the gel cast, with a comb, to solidify. Into each comb well of the agarose gel, a mixture of 12 µl of nested PCR product and 2 µl TrackIT™ Cyan/Yellow Loading Buffer (Invitrogen) was added. In the first well of each row, a ladder was run (a mixture of 1 µl TrackIT™ 1 Kb Plus DNA Ladder (Invitrogen), 2 µl TrackIT™ Cyan/Yellow Loading Buffer, and 11 µl DEPC-Treated Water). The PCR amplification products were purified using QIAquick PCR Purification Kit (Qiagen), following manufacture's recommended procedure.

2.4.4 Sequencing of Purified PCR Products

Bidirectional sequencing of the nested PCR amplicons using AML1 and AML2 was performed with the BigDye 3.1 Terminator cycle sequencing kit (Life Technologies, Carlsbad, CA) using a Life Technologies 3730 DNA Analyzer as per the manufacturer's recommended procedure.

2.4.5 Identification of Spore Genus

Sample DNA sequences were trimmed and aligned using Sequencher version 5.2.4. Software (Gene code Corporation, Ann Arbor, MI USA). The resulting contig for each sequence was entered into the MaarjAM Database (Öpik et al., 2010), for identification of the fungal taxon by the BLAST search alignments. Using the INVAM classification page (INVAM 2013), the most probable sequence matches were compared to the morphological characteristics of the spore (size, color, etc.), narrowing down which genus the spore belonged to. For each olive cultivar, a percentage of spores belonging to each specific genus were determined.

2.4.6 Statistical Analysis

Statistical analysis on the means of replicate growth experiments were performed using the ANOVA procedure of the InStat® software, 230 version 3.0 (GraphPad, San Diego, CA) and the Tukey's test of significance between means. Significance was reported if the P-value was at least < 0.05. The same software was used for chromatographic analysis of triplicate extract samples with triplicate injection using unpaired a T test.

3. Results and Discussions

3.1 The Effect of AM Fungus Communities from Different Olive Cultivars on the Growth of Leeks

AM fungi from the rhizosphere of different olive cultivars, were first multiplied in trap cultures, then a portion of the trap culture was used to inoculate leek seedlings to compare the effect of different AM fungi communities on plant growth. In parallel, control leek plants were grown using the same soil mix, but autoclaved to destroy mycorrhizae. All AM fungus communities significantly increased the growth of the leek plants both by plant height and shoot dry weights (Table 1). It is however, interesting to note that mycorrhizal fungi from Frantoio and Manzanillo cultivars gave significantly superior growth response than the AM fungi communities mycorrhizae obtained from Mission cultivar (Table1).

Table 1. The growth of leeks inoculated with trap culture soils containing AM fungus communities originally found in five different olive cultures compared to uninoculated control leeks

Treatment	Plant Height (cm)	Dry Shoot Weight (g)
Frantoio	25.60 ± 1.2	0.216 ± 0.013
Koroneiki	22.20 ± 0.6	0.174 ± 0.006
Arbequina	23.90 ± 0.7	0.192 ± 0.012
Mission	21.40 ± 0.4	0.118 ± 0.022
Manzanillo	25.30 ± 0.6	0.188 ± 0.016
*Control	16.60 ± 0.8	0.081 ± 0.010
**Notable Differences	Frantoio > Mission	Frantoio > Mission
	Manzanillo > Mission	Manzanillo > Mission

Note. Values in the table are means of six observations. * In all cases, the inocula from all the olive cultivars were significantly greater than the control. ** In the notable differences section the > means that the cultivar to the left were significantly greater than the cultivar to the right of the sign.

Communities of AM fungi associated with Frantoio and Manzanillo that gave superior results in terms of plant growth and development in leeks happened to contain highest number of AM fungus genotypes mycorrhizae genera belonging to the family Gigasporaceae (42 and 56 percent among total number of spores respectively) (Table 2A). The genus *Gigaspora* was uniquely associated with Manzanillo and no other cultivar (Table 2B). The dominant genus associated with Frantoio was *Scutellospora*, but unlike *Gigaspora*, this genus is not unique to Frantoio as it occurred in modest amounts associated with other cultivars used in this study (Table 2B). The only genus that was uniquely associated with Frantoio is *Septoglomus*, but it occurs in small amounts (1.5%), and, therefore, it would be unlikely that the superior impact on growth from inoculum with Frantoio could be attributed to this genus (Table 2B). Inoculum spores multiplied from rhizosphere soil of cv Mission tend to give the poorest growth promoting response on leeks (Table 1). This inoculum was dominated by genus *Rhizophagus* (41%) (also in Arbequina, and Koroneiki), which occurred the least(4.2%) in the high performer cultivar Frantoio and in modest amounts (14.8%) in Manzanillo (Table 2B). Based on the discussion above and data presented in Table 2, a pattern seems to emerge indicating that good performing inocula were dominated by *Scutellospora* (33.3%) in inoculum from Frantoio and by *Funneliformis* and *Gigaspora* (29.6 and 25.9 percent respectively) in Manzanillo. It is notable that the best performing inocula from Frantoio had the greatest diversity of mycorrhizae families, but the next best performing inoculum from Manzanillo had the least diversity (Table 2 A). Thus, it appears the better performance was due to presence of dominant species and not due to diversity. The poor or modest performing inocula were dominated by genus *Rhizophagus*, which occurred 40.7% in Mission, 36.8% in Arbequina, and 51.9% in Koroneiki (Table 2A). Further trap cultures are now being prepared, using isolated spores, to identify the species. Further tests will be performed to identify super performers, not only with leeks, but also with olive cultivars.

Table 2. Percentage of spores from olive cultivars found in different mycorrhizal genus/family

A. Percentage of spores from olive cultivars in different mycorrhizal families

Family	Frantoio	Mission	Arbequina	Manzanillo	Koroneiki
Glomeraceae	30.56%	54.24%	57.89%	44.44%	75.00%
Pacisporaceae			2.63%		
Acaulosporaceae	1.39%		5.26%		
Diversisporaceae		3.39%			
Gigasporaceae	41.67%	32.20%	28.95%	55.56%	23.08%
Claroideoglomeraceae	8.33%				
Paraglomeraceae	4.17%	6.78%			
Archaeosporaceae	1.39%		2.63%		
Ambisporaceae	12.50%	3.39%	2.63%		1.92%

B. Percentage of spores from olive cultivars in different mycorrhizal genera

Family	Genus Types	Frantoio	Mission	Arbequina	Manzanillo	Koroneiki
Glomeraceae	*Funneliformis*	5.56%	8.47%	13.16%	29.63%	23.08%
	Septoglomus	1.39%				
	Glomus	19.44%	5.08%	7.89%		
	Rhizophagus	4.17%	40.68%	36.84%	14.81%	51.92%
Pacisporaceae	*Pacispora*			2.63%		
Acaulosporaceae	*Acaulospora*	1.39%		5.26%		
Diversisporaceae	*Diversispora*		3.39%			
Gigasporaceae	*Gigaspora*				25.93%	
	Dentiscutata	8.33%	13.56%	13.16%	16.67%	7.69%
	Scutellospora	33.33%	18.64%	15.79%	12.96%	15.38%
Claroideoglomeraceae	*Claroideoglomus*	8.33%				
Paraglomeraceae	*Paraglomus*	4.17%	6.78%			
Archaeosporaceae	*Archaeospora*	1.39%		2.63%		
Ambisporaceae	*Ambispora*	12.50%	3.39%	2.63%		1.92%

3.2 Identification and Quantitative Analysis of Polyphenols in Leeks Grown with Mycorrhizae Inocula Obtained from the Soils of Different Olive Cultivars

3.2.1 Separation and Identification of Polyphenols in Leek Shoots

The polyphenol peaks from leek extracts whose levels increased or decreased with different AM fungi inoculations compared to the control, are identified by numbers in Figure 1.

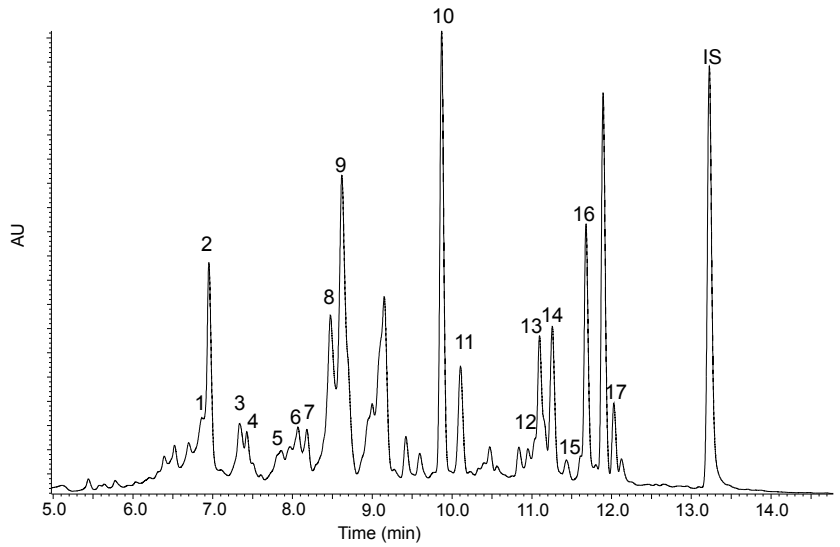

Figure 1. Separation of different polyphenols extracted from leek shoots on HPLC column.

Note. The polyphenol derivatives associated with the peaks are listed in Table 3.

Most of the identified products have quercetin (peaks #1, #5, #11, and #14) and kaempferol (peaks # 2-4 and #7-10) as was previously reported (Malik et al., 2015) but also an aglycone with a possible molecular formula $C_{15}H_{10}O_5$ can be identified in the spectra of the peaks #12 and #13, (Table 3), the mass determination of these two peaks was affected by a poor ionization of the products causing a larger error in the $[M+H]^+$ ion, but the MS/MS fragment corresponding to the aglycone seem to be consistent with the formula proposed. A literature search indicated that this formula matched with apigenin, a common flavone associated with polyphenols, and we are proposing that structure for the corresponding product (Hermann, 1988).

Table 3. Polyphenol derivatives associated with the peak # in the chromatogram from Figure 1.

Peak # & RT[a]	Aglycone	$[M+H]^+_{Cal.}$[b]	$[M+H]^+_{Exp}$[b]	Pent[c]	Hex[c]	HexA[c]	Malonyl	Ferul.[c]	Coumaroyl
1 (6.89)	Quercetin	875.2088	875.2090		3		1		
2 (6.95)	Kaempferol	1021.2670	1021.2670		4		1		
3 (7.34)	Kaempferol	873.1931	873.1937		2	1	1		
4 (7.43)	Kaempferol	859.2139	859.2131		3		1		
5 (7.97)	Quercetin	1127.2933	1127.2940		4	1			
6 (8.07)	Unknown	N/A	1213.3008						
7 (8.18)	Kaempferol	1359.3669	1359.3622		5		1	1	
8 (8.48)	Kaempferol	1197.3140	1197.3163		4		1	1	
9 (8.62)	Kaempferol	1167.3015	1167.3040		4		1		1
10 (9.87)	Kaempferol	1229.3614	1229.3624	1	5				
11 (10.11)	Quercetin	1245.3563	1245.3578	1	5				
12 (10.95)	Apigenin	1243. 3770	1243.5848		6				
13 (11.10)	Apigenin	1213.3665	1213.5814	1	5				
14 (11.26)	Quercetin	889.1881	889.1840		2	1	1		
15 (11.44)	Unknown	N/A	1227.6013		6				
16 (11.68)	Unknown	N/A	1197.5889	1	5				
17 (12.03)	Unknown	N/A	1033.5569	1	3				

Note. [a] Retention time (RT) in minutes;

[b] $[M+H]^+_{cal}$ is the calculated exact mass of the protonated molecule and $[M+H]^+_{Exp}$ is the experimental mass from the Q-TOF analysis of the polyphenols;

[c] Pen = pentose, Hex= hexose, HexA = hexuronic acid, and Ferul = feruloyl.

However, we are not aware of this flavone being reported before in leek and this finding will require further investigation. In addition, the MS/MS of the corresponding products provided information for identification of the *O*-glycosylation (pentose (Pent), hexose (Hex), and hexuronic acid (HexA)) and other moieties associated with the polyphenol. The $[M+H]^+$ calculated and the experimental mass obtained with the Q-TOF mass spectrometer are also reported in Table 3 showing the low instrument error to support the identified polyphenols. We are not providing specific structures here for the products shown in Table 3 because the limitation of the mass spectrometry to provide the information necessary for the full characterization of the type of sugar and linkage position; however, we have showed before (Malik et al., 2015) the structures for peak #2, 8, and 9 based on the work of Di Donna et al., 2014. Unfortunately peak # 6, and 15-17 do not have sufficient information to identify the associated aglycone and are reported as unknowns.

3.2.2 Quantitative Response of Polyphenol Levels to Mycorrhizae Inocula Obtained from Different Olive Cultivars

In extracts from leeks inoculated with mycorrhizae obtained from cv Frantoio roots, the majority of polyphenols showed increased levels compared to un-inoculated controls (Table 4A). In fact, the majority of polyphenols in the extracts of leeks inoculated with mycorrhizae from Frontoio were higher (except peaks #3, #4 and #11) compared with polyphenol profiles of leek extracts from plants inoculated with AM fungi from rhizosphere soils of other olive cultivars (Table 4B). Similarly, extracts from leeks inoculated with mycorrhizae from roots of Manzanillo trees showed higher levels of different polyphenols compared to extracts from leeks that were inoculated with mycorrhizae from other cultivars, except Frantoio (Table 1A). Inoculum from the roots of Mission cultivar, that gave poorest plant growth (as noted above), also showed lower polyphenols compared to other cultivars (Table 4C). These studies indicate that inoculation of leeks with mycorrhizal fungi obtained from the roots of any olive cultivar has a positive effect on growth and development of leeks compared to control plants without mycorrhizae. However, mycorrhizal inoculum prepared from cvs Frantoio and Manzanillo gave significantly higher growth than leek plants inoculated with AM fungi obtained from cv Mission. The better performing inocula were dominated by mycorrhizal fungi of the family Gigasporaceae: the genus *Scutellospora* was more prevalent in inoculum from cv Frantoio, while the inoculum from cv Manzanillo contained more of the genus *Gigaspora*. Mycorrhizal fungus colonization increased the majority of polyphenol species in leeks

compared to uninoculated controls. AM fungus inoculum from cv Frantoio produced higher levels of polyphenols in leeks while inoculum from Mission had lowest levels of polyphenols compared to inocula from other cultivars. The studies described here provide information that selection of mycorrhizal communities from the roots of different plant cultivars is a valuable method to enhance growth and quality (increase polyphenol levels) of produce of various crops. Polyphenols are known for their powerful health benefits, and therefore, an increase in their levels would increase the marketability of the produce.

Table 4A. Percent change in the levels of individual polyphenols, as described by Retention Time in HPLC profile, in leeks when inoculated with mycorrhizae from different olive cultivars compared to uninoculated controls

	6.86	6.95	7.34	7.43	7.97	8.07	8.18	8.48	8.62	9.87	10.11	10.95	11.1	11.26	11.44	11.68	12.03
Soil Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Frantoio	NS	54	-62	-30	161	423	161	233	130	11	-26	75	52	68	100	86	125
Koroneiki	-16	NS	-40	NS	NS	68	-29	71	NS	-22	43	34	NS	28	81	74	97
Arebequina	-56	-34	-77	-52	NS	136	NS	86	19	-44	-62	NS	-32	-52	NS	26	35
Mission	-66	-61	-74	-53	NS	68	-41	206	NS	-48	-62	NS	37	38	38	36	67
Manzanilla	-37	-24	-66	-18	23	104	29	198	43	NS	-47	37	45	59	112	133	186

Note. Green color indicates a significant increase in polyphenol when inoculated with mycorrhizae from an olive cultivar; Red color indicates a significant decrease in polyphenol when inoculated with mycorrhizae from an olive cultivar; NS indicates there was no significant difference between polyphenol levels.

Table 4B. Percent change in levels of individual polyphenols in leeks inoculated with mycorrhizae taken from olive cultivar Frantoio compared to leeks inoculated with mycorrhizae from other cultivars

	6.86	6.95	7.34	7.43	7.97	8.07	8.18	8.48	8.62	9.87	10.11	10.95	11.1	11.26	11.44	11.68	12.03
Soil Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Koroneiki	NS	48	-38	-28	175	207	264	93	144	41	-48	30	50	31	10	6	13
Arebequina	NS	137	69	47	165	98	175	81	95	98	96	NS	125	252	NS	48	68
Mission	NS	240	NS	NS	111	162	273	NS	87	80	67	46	NS	NS	24	NS	NS
Manzanilla	NS	103	NS	NS	110	155	100	11	61	NS	41	27	4	6	-5	-20	-21

Note. Green color indicates a significant increase in polyphenol when inoculated with mycorrhizae from Frantoio; Red color indicates a significant decrease in polyphenol when inoculated with mycorrhizae from Frantoio; NS indicates there was no significant difference between polyphenol levels.

Table 4C. Percent change in levels of individual polyphenols in leeks inoculated with mycorrhizae taken from olive cultivar Mission compared to leeks inoculated with mycorrhizae from other cultivars

	6.86	6.95	7.34	7.43	7.97	8.07	8.18	8.48	8.62	9.87	10.11	10.95	11.1	11.26	11.44	11.68	12.03
Soil Type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Frantoio	NS	-75	NS	NS	-59	-67	-77	NS	-54	-52	-49	-41	NS	NS	-31	NS	NS
Koroneiki	-59	-63	-57	-51	11	NS	-17	79	11	-33	-74	-23	36	8	-24	-22	-16
Arbequina	-22	-41	15	-2	6	-36	-38	65	-12	-7	NS	NS	101	186	NS	8	24
Manzanillo	-45	-49	-24	-43	-15	-17	-54	3	-27	NS	-28	-25	-5	-13	-35	-41	-42

Note. Green color indicates a significant increase in polyphenol when inoculated with mycorrhizae from Mission; Red color indicates a significant decrease in polyphenol when inoculated with mycorrhizae from Mission; NS indicates there was no significant difference between polyphenol levels statistical analysis were performed as described in Materials and Methods.

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