Biology and Fertility of Soils (2024) 60:115–136
https://doi.org/10.1007/s00374-023-01778-6

ORIGINAL PAPER

Arbuscular mycorrhizal fungi with contrasting life-history strategies differently affect health-promoting compounds in field-grown tomato by changing arbuscule occurrence and mycorrhizal assemblages in roots

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Received: 30 May 2023 / Revised: 6 November 2023 / Accepted: 8 November 2023 / Published online: 24 November 2023
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Abstract

Contrasting life-history characteristics of arbuscular mycorrhizal (AM) fungal families may have important implications for mycorrhizal functioning. Nevertheless, the effect of inoculation with AM fungi having different life-history strategies on the quality parameters of tomato fruits was not investigated. In this study, fruit and sauce quality of two tomato varieties were evaluated in field conditions after inoculation with four AM fungal species belonging to Glomeraceae and Gigasporaceae. The functional relationship between AM fungal traits (i.e., root colonization structures, community diversity) and fruit quality parameters was analyzed. AM fungal inoculation increased total phenols (TPC) and lycopene concentration in fruits of both varieties (47% and 247%, respectively) and antioxidant activity in var. Rio Grande (85%). Gigasporaceae were more effective in increasing TPC and antioxidant activity compared to Glomeraceae in var. Rio Grande. *Gigaspora gigantea* outperformed *Scutellospora pellucida* in var. Pisanello for TPC, antioxidant activity, and lycopene. Inoculated strains of *G. gigantea*, *S. pellucida*, *Funneliformis mosseae*, and *Sclerocystis sinuosa* were molecularly retrieved within tomato roots. In both varieties, a functional relationship between occurrence of arbuscules in roots and fruit quality was found. In var. Rio Grande, the abundance of some native AM fungal taxa shaped the pattern of fruit quality parameters. Gigasporaceae might be of great relevance for the synthesis of health-promoting compounds in tomato and should be included in biostimulant programmes targeting the production of high-quality vegetables.

Keywords Arbuscular mycorrhizal fungi · Food quality · Lycopene · Antioxidants · Functional groups · Community diversity

Introduction

Arbuscular mycorrhizal fungi (AMF) belonging to Glomeromycota phylum (Hibbett et al. 2007; Schüβler et al. 2001; Tedersoo et al. 2018) are largely applied in horticulture as microbial biostimulants since they can lower the consumption of mineral fertilizers, as well as improve their use efficiency (Bender and van der Heijden 2015; Cavagnaro et al. 2015; Schütz et al. 2018; De Santis et al. 2022). Moreover, AMF can maintain or even enhance plant growth and food quality. In the strongly competitive fresh food market, in which greenhouse and field growers need to meet the high requests of consumers, nutritional quality and health benefits of vegetables are becoming increasingly important (Dias et al. 2012; Fanasca et al. 2006; Kyriacou and Rouphael 2018). In this context, AMF could play a pivotal role as they were reported to boost the production of health-related compounds in several vegetables, such as lettuce (*Lactuca sativa* L.) (Avio et al. 2017; Baslam et al. 2011a, b; 2013a, b), tomato (*Solanum lycopersicum* L.) (Aguilera et al. 2022; Bona et al. 2017; Carillo et al. 2020; Hart et al. 2015), pepper (*Capsicum annuum* L.) (Mena-Violante et al. 2006), artichoke (*Cynara cardunculus* L.), green asparagus (*Asparagus officinalis* L.) (Conversa et al. 2019), onion (*Allium cepa* L.)

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(Golubkina et al. 2020), basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.) (Saleh et al. 2020), and eggplant (*Solanum melongena* L.) (Sabatino et al. 2020). However, the output of the symbiosis may depend on factors, such as plant variety, AM fungal identity, agronomic practices, and interaction with other beneficial microorganisms and abiotic stresses (e.g. Baslam et al. 2011a,b, 2012; Baum et al. 2015; Bona et al. 2018; Carillo et al. 2020; Copetta et al. 2011; Mena-Violante et al. 2006; Saleh et al. 2020).

Tomato is one of the major vegetables in the world in term of production (FAOSTAT database, average period 2011–2020; https://www.fao.org/faostat/en/#data/QCL). It has assumed the status of "functional food" due to its high concentration in health-related biomolecules (nutraceuticals) (Dorais et al. 2008) that have been proven to reduce risk of cancer and cardiovascular diseases upon consumption (e.g., Agarwal and Rao 2000; Giovannucci 1999; Heber 2000; Toor and Savage 2005). This protective effect has been mainly attributed to the antioxidant activity performed by the carotenoid constituents of the fruits, particularly lycopene and β-carotene (Clinton 1998; Di Mascio et al. 1989; Stahl and Sies 1996). Other bioactive compounds abundant in tomato fruits are ascorbic acid and vitamin E, as well as polyphenols and terpens (Emmanuel and Babalola 2020; Gruda 2005).

The effect of AM fungal inoculation on tomato fruit quality was investigated mostly in controlled conditions, i.e., in pots in greenhouse and using single AM fungal species (i.e., *Funneliformis mosseae* and *Rhizophagus intraradices*) (Giovannetti et al. 2012; Hart et al. 2015; Nzanza et al. 2012; Ulrichs et al. 2008; Zouari et al. 2014) or mixtures of AMF from two to five species (Aguilera et al. 2022; Chouyia et al. 2022; Copetta et al. 2011; Hart et al. 2015). In such conditions, some significant increases of lycopene concentration (from 12 to 125%) (Aguilera et al. 2022; Copetta et al. 2011; Giovannetti et al. 2012; Ulrichs et al. 2008) and total phenols (9%) (Ulrichs et al. 2008) were detected in fruits. Enhanced levels of β-carotene were observed in fruits of tomato plants treated with single-inoculum (+45%) (Ulrichs et al. 2008) and with mixture of two AM fungal species (+27%) (Hart et al. 2015). Moreover, increases of total antioxidant activity in fruits of tomato inoculated with *F. mosseae* was only detected by Zouari et al. (2014) (+51%). The increases of secondary metabolites in tomato plants inoculated with *F. mosseae* are also supported by the results of transcriptomic analyses (Fiorilli et al. 2009; Salvioli et al. 2012; Zouari et al. 2014).

Under field conditions, where native AM fungal communities compete with inoculated AMF, the response pattern of fruit content of health-related compounds in tomato due to mycorrhizal inoculation is not clear (Bona et al. 2017, 2018; Carillo et al. 2020; Njeru et al. 2017; Pasković et al. 2021; Subramanian et al. 2006). Lycopene was variably enhanced in fruits of inoculated plants (from 5 to 47%) (Bona et al. 2017; Carillo et al. 2020), whereas was unchanged in other studies (Njeru et al. 2017; Pasković et al. 2021). Moreover, recently, increases of lipophilic and hydrophilic antioxidant activity (12% and 9%, respectively) and hydrophilic total phenols (8%) were reported in fruits of inoculated plants (Carillo et al. 2020; Pasković et al. 2021). However, these studies did not investigate the functionality of isolates belonging to different families of AMF or the changes in roots of the AM fungal communities following inoculation.

Life-history characteristics of AMF belonging to different families, such as Gigasporaceae and Glomeraceae, may have important implications for mycorrhizal functioning (e.g., Arcidiacono et al. 2023; Brundrett et al. 1999; Hart and Reader 2002, 2004; Klironomos and Hart 2002; Merryweather and Fitter 1998). Members of Gigasporaceae, extensively exploring the soil by hyphae, could give a boost to host plant growth and nutrient uptake (Chagnon et al. 2013; de Souza et al. 2005; Hart and Reader 2002; Maherali and Klironomos 2007). On the opposite side, members of Glomeraceae family, intensively colonising the root system, could have a beneficial effect on the suppression of root pathogens with a consequent strong benefit on plant growth and fruit yield. Arcidiacono et al (2023) found that tomato plants of var. Pisanello and var. Rio Grande inoculated with members of Gigasporaceae produced fruits with higher concentration of N, K, Zn, Cu, Mg, and Mn respect to those inoculated with Glomeraceae. However, there is no data available on the impact of the inoculation with AMF with different life-history strategies on the quality parameters of tomato fruits. So far, inoculation experiments were not supported by reliable molecular identification of the AM fungal taxa in roots. Indeed, in the field, the presence of the native AMF, competing with the inoculated AM fungal isolates, could have important implications on the output of the mycorrhizal symbiosis (e.g., Ercoli et al. 2017; Pellegrino et al. 2012, 2022).

The aim of this study was to investigate under field conditions how the inoculation of tomato with four single AM fungal isolates belonging to Glomeraceae and Gigasporaceae can affect the nutraceuticals content of fruits and related transformed products, such as tomato sauce. We selected two varieties of tomato, characterized by different growth habits: var. Pisanello (an old local variety) with an indeterminate growth habit and var. Rio Grande (a widely used modern variety) with a determinate growth habit. We set-up two field experiments: one in 2019 with var. Pisanello and one in 2020 with var. Rio Grande. Two AM fungal species per family were chosen to provide replication. We hypothesized the existence of functional trade-offs among AM fungal species belonging to Gigasporaceae and Glomeraceae and correlated functional traits within members of each family (Chagnon et al. 2013; Maherali and Klironomos 2007). Members of

Gigasporaceae, extensively exploring the soil by hyphae, were figured to have a stronger effect on fruits nutrient uptake and consequently on the content of health-related secondary metabolites, such as lycopene, total phenols, and antioxidant activity, in comparison with members of Glomeraceae that extensively colonize the root system. We also hypothesized that development of AM fungal taxa within roots is affected by the competition between fungal species belonging to native community and inoculated species and differences at family level would be related to changes of diversity of AMF in roots, due to the expected higher colonization ability of Glomeraceae. Finally, we expected that both functional fungal traits (i.e., AM fungal abundance and community diversity) would have been highly correlated with quality parameters (i.e., lycopene, total phenols, and antioxidant activity) in tomato fruits.

Materials and methods

Characterization of the field site

The field experimental sites were located at the organic farm "Fattoria Le Prata" (Pisa, Italy) (43°44′ N, 10°24′ E; 2 m above sea level and 0.0% slope). The soil of the experiment, carried out in 2019, was a silty-clay loam (8.0% sand, 54.1% silt and 37.9% clay) 7.9 pH (deionized water 1:2.5 w/v; McLean 1982), 2.2 g kg^{-1} total N (Kjeldahl; Bremner and Mulvaney 1982), 1.89 g kg^{-1} total P, and 22.00 mg kg^{-1} available P (Olsen; Olsen and Sommers 1982), and with 35.9 g kg^{-1} soil organic C (SOC) (Walkley–Black; Nelson and Sommers 1982). The soil of the experiment, carried out in 2020, was a silty-clay loam (10.2% sand, 50.2% silt, and 39.6% clay), 8.0 pH (H_2O), 1.83 g kg^{-1} total N (Kjeldahl), 1.72 g kg^{-1} total P, and 17.0 mg kg^{-1} available P (Olsen), with 30.5 g kg^{-1} soil organic C (Walkley–Black). Climate of the field area is cold, humid Mediterranean (Csa), according to the Köppen-Geiger climate classification (Kottek et al. 2006). From May to September 2019, corresponding to the field growth cycle of tomato, maximum and minimum temperatures were 27.4 °C and 16.0 °C, respectively, and total precipitation was 236 mm. From May to September 2020, maximum and minimum temperatures were 27.4 °C and 15.5 °C, respectively, while total precipitation was 270 mm. The preceding crop of tomato grown in 2019 and 2020 was bread wheat.

Fungal and plant material

The AMF used as inocula were *Gigaspora gigantea* PA125 and *Scutellospora pellucida* MN408A belonging to Gigasporaceae, and *Funneliformis mosseae* MD118 and *Sclerocystis sinuosa* MD126 belonging to Glomeraceae. Inocula were obtained from pot cultures maintained in the collection of the Crop Science Research Center of the Scuola Superiore Sant'Anna, Italy. Details about inocula are given in Table S1. The plant material used in 2019 and 2020, respectively, were *Solanum lycopersicum* L. var. Pisanello, an old Tuscan variety described and conserved in the regional genetic bank for the conservation of endangered (or threatened) varieties (http://germoplasma.regione.toscana.it/; Berni et al. 2018), and *S. lycopersicum* L. var. Rio Grande, a modern widely used variety.

Tomato inoculation with AMF at the nursery

Inoculation by AMF was performed in a climatic chamber at the sowing (var. Pisanello: 11th March 2019; var. Rio Grande: 15th April 2020), before transplanting the plantlets to the field. In detail, 160 seeds of *S. lycopersicum* were placed in a propagation tray with hole dimension of 26.4 mL (total volume: 4.22 L), containing as substrate a mixture (1:1:2:2 by volume) of peat, soil, coarse silica sand and heat-expanded clay. The soil used in the mixture was a sandy loam collected at the "Centro di Ricerche Agro-Ambientali Enrico Avanzi," University of Pisa, Italy. Soil chemical and physical characteristics were 8.0 pH(deionized water 1:2.5 w/v; McLean 1982), 15.3% clay, 30.1% silt, 54.5% sand, 2.2% organic matter (Walkley–Black; Nelson and Sommers 1982), 1.3 g kg^{-1} total N (Kjeldahl; Bremner and Mulvaney 1982), 469.5 mg kg^{-1} total P (Olsen), 17.6 mg kg^{-1} extractable P (Olsen) (Olsen and Sommers 1982), and 149.6 mg kg^{-1} extractable K (Thomas 1982). The mixture was steam-sterilized (121 °C for 25 min, on two consecutive days) to kill naturally occurring AMF. The tray was inoculated either with 850 mL of crude inoculum (mycorrhizal roots and soil containing spores and extraradical mycelium) of one out of the four AMF or with 850 mL of a sterilized mixture of them (control). The non-mycorrhizal control was steam-sterilized (121 °C for 25 min, on two consecutive days). A high amount of inoculum (10% by volume) was used to balance potential differences in AM fungal colonization ability of the four inocula. Each tray received 565 mL of a filtrate (45-μm mesh size), obtained using a mixture of the four crude inocula and of a sample of the agricultural soil used in the mixture, to ensure to all treatments a common prokaryotic community. Plants were grown in a climatic chamber from the start of April to the end of May on both the years of cultivation (2019 and 2020) (24 °C and 18 °C night temperature; 14:10 h light:dark cycle, 420 μmol m^{-2} s^{-1}) (ca. 60 days) and were supplied with tap water as needed and with a half-strength Hoagland solution every month (70 mL per tray).

Experimental set-up

Tomato plantlets (mean length of plantlets: 18 cm) of var. Pisanello were transplanted into the field in May (60 plants per replicate plot for var. Pisanello on16th of May 2019; 32 plants per replicate plot for var. Rio Grande on 23rd of May 2020) (a total of 900 and 480 plants, respectively). The size of the field in 2019 was 1248 m^2 (52-m length × 24-m width), while the size of 2020 was 1350 m^2 (90-m length × 15-m width). The infectivity of the soils before transplating was very low and similar between years (e.g., 9.1% of AM fungal root colonization assessed by the mycorrhizal infection potential test; Arcidiacono et al. 2023). A completely randomized design with five levels of the treatment AM fungal inoculation (Inoc) (*G. gigantea*, *G.giga*; *S. pellucida*, *S.pellu*; *F. mosseae*, *F.mos*; *S. sinuosa*, *S.sin*; mock-inoculated control, – M) was set up in 2019 and 2020 for var. Pisanello and Rio Grande, respectively. Schematic overview of the experimental design is given in Arcidiacono et al. (2023). Each level of Inoc was replicated three times. The replicate plot of the experiment with var. Pisanello had a size of 9.6-m length × 1.6-m width (15.4 m^2) and was composed by two rows with 30 plants for each row (30 cm within the rows and 1 m between rows). Each replicate plot of the experiment with var. Rio Grande had a size of 2.5-m length × 1.25-m width (3.1 m^2) and was composed by four rows with eight plants for each row (25 cm within and between the rows). In both years, the plots were separated by uninoculated plantlets of the same variety. The tomato plants were daily watered through drip irrigation, with pressure regulation, allowing similar water flow to each plot. No mineral fertilization neither chemical nor mechanical weed control were applied.

Sampling of fruits for quality assessment and of roots for molecular analysis

Harvests were performed at BBCH 89 (fully ripe: all fruits have typical fully-ripe color) (Meier 2001) on 10 plants in the central area of each plot to avoid potential border effect. Plants of var. Pisanello were harvested two times: 22nd July and 1st of August 2019. Similarly, plants of var. Rio Grande were harvested two times: 3rd August and 10th August 2020. All sampled fruits showed a ripeness stage 6 (red) (USDA 1975). Tomato fruits were washed and immediately stored at – 80 °C. Then, fruit samples were lyophilized and ground to a fine powder prior to the extraction and determination of total phenolic content (TPC), antioxidant activity, and lycopene content. In each experiment, in order to cover AM fungal spatial variability, five soil cores per each replicate plot were collected at 0–20-cm soil depth and then pooled in a combined soil sample. Sampling was carried out for both varieties at the 2nd harvest (BBCH 89; 1st of August 2019 and 10th August 2020, respectively). From each combined soil sample. roots were manually collected with forceps and washed by wet-sieving and decanting down to a mesh size of 250 mm. After removing organic debris, live and fine roots were carefully plucked with forceps and stored at – 80 °C for further analysis. Moreover, for var. Rio Grande, an additional root sampling was carried out when plantlets were transplanted in the field (BBCH 22; 23rd May 2020).

Fruit qualitative analyses

Extraction for TPC and antioxidant activity determination was done as described by Morra et al. (2021) with minor modifications. In detail, approximately 4 g of lyophilized sample (in duplicate) was homogenized in 20–25 mL of 80:20 CH_3OH/H_2O mixture and sonicated in an ultrasonic bath (Elmasonic P Elma, Singen, Germany) at 30 °C for 3 h into the darkness. Afterwards, the extracts were filtered and the flow through was taken and kept at – 20 °C until further analyses. The TPC was determined according to Folin–Ciocalteu method (Singleton and Rossi 1965) with minor modifications. The tube test contained 1 mL H_2O, 100 µL of tomato extract and 100 µL of Folin-Ciocalteu phenol reagent 2N solution and, after 10 min, 800 µL of Na_2CO_3 75 g L^{-1} solution. The mixture was kept for 120 min at room temperature into the darkness, and the absorption was measured at 765 nm against a reagent blank. Measurements were performed in duplicate, and results were expressed as mg of gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} d.w.). For calibration, standard solutions of gallic acid (range 0–150 µg mL^{-1}) were prepared and estimated as above.

The antioxidant activity of fruit samples was measured using the oxygen radical absorbance capacity (ORAC) assay kit (Cell Biolabs Inc., USA). The reaction was performed according to the protocol developed by Cao et al. (1993) and validated by Cao and Prior (1999). The antioxidant standard curve was prepared using the water-soluble vitamin E analog TroloxTM. Standards (25 µL) and tenfold diluted samples (25 µL) were added into the wells of a microplate (black 96-well plates, NuncTM black microwell, Japan). Fluorescein solution, made by diluting the Fluorescein Probe 1:100 with 1X Assay Diluent, was rapidly added to each well (150 µL) using a multichannel pipette, and the plate was incubated for 30 min at 37 °C. Afterwards, Free Radical Initiator Solution (25 µL) was added into each well and the plate was immediately placed in a fluorescence microplate reader (Wallac Victor 3 Multilabel Plate Reader, PerkinElmer Inc., Waltham, MA). The fluorescence intensities were recorded every 5 min for 60–90 min at 37 °C with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Results, calculated according to the kit protocol, were

expressed in μMol Trolox equivalent antioxidant capacity per g of dry weight (μMol TE g^{-1} d.w.).

The quantification of lycopene (ψ,ψ-carotene), was performed according to Daood et al. (2014). The samples were dissolved in acetone and n-hexane (2:1, *v*/*v*), centrifugated and filtered through a 0.45-μm pore size nylon Millipore. The extraction was performed under dark conditions to avoid lycopene isomerization and degradation. LC–MS for metabolomic analysis was performed using UHPLC-MS (Xevo TQ Absolute Triple Quadrupole Mass Spectrometry, QCA1743, Waters, Milford, MA, USA), and an electrospray ionization (ESI) probe that was operated in the positive ion mode to detect lycopene. An ACQUITY UPLC BEH C18 analytical column (2.1 mm × 100 mm, 1.7 μm particle size) was used for the analysis of the analyte present in each sample, using a column temperature of 35 °C and a flow rate of 0.8 mL/min. The solvent mixture consisted of water (solvent A) and acetone (solvent B). The gradient elution program was set as follows: 0.0–1.5 min, 80% B and 20% A; 1.5–2.5 min, 88% B and 12% A, 2.5–3.5 min, 95% B and 5% A, and finally 80% B and 20% A. The injection volume was set at 2 μL. MS parameters were as follows: the capillary voltage was set at 3.4 kV, while blocking and desolvation temperatures were set at 150 °C and 450 °C, respectively. The desolvation gas flow rate was set at 700 L/h, and the cone gas was set at 50 L/h. Cone voltages were set to 75 V, and collision energies were set to 20 eV.

Transformation of fresh tomato into tomato sauce and qualitative analyses

The tomato sauce was produced in a pilot plant at the "Fattoria Le Prata" (Pisa, Italy) from the tomato fruits collected at the 2nd harvest. Tomato samples from three replicates per each treatment were subjected to the following processing steps: (i) homogenization and blanching at 80 °C for 3 min in a stainless-steel pot; draining and passing through a sieve (hole diameter of 8 mm); filling in sterilized glass jar (volume of 500 mL); pasteurization at 92 °C for 2 min. For both varieties, the fruit qualitative analyses (TPC, antioxidant activity, and lycopene) were carried out on the tomato sauce obtained from the transformation of the fresh tomatoes.

Extraction of genomic DNA, PCR amplification, cloning, and sequencing of AM fungal community in roots of tomato

Three genomic DNA extractions from roots were performed per each replicate plot (roots were obtained from the combined soil sample obtained from each replicate plot) (Renker et al. 2006). Roots were collected at the BBCH 89 growth stage (2nd inflorescence with first flower open; Meier 2001) in both varieties as well as at BBCH 22 (2nd primary apical side shoot visible) in var. Rio Grande. Each extraction was performed from a subsample of 20 mg root dry weight using the Dneasy® Plant Mini Kit (Qiagen, Germantown, MD, USA). For var. Pisanello 45 DNA extractions were performed (three technical replicates x five levels of treatment Inoc x three plot replicates) and for var. Rio Grande 45 DNA extractions were performed at transplanting (three technical replicates x five levels of treatment Inoc x three plant replicates) and 45 DNA extractions were performed at harvest (three technical replicates x five levels of treatment Inoc x three plot replicates). From each DNA extract, a PCR amplification was performed using the primer pair the SSUmAf–LSUmAr, targeting part of the SSU, the complete ITS region (including the 5.8S rRNA gene), and approx. 0.8 kb of the LSU rRNA gene (Krüger et al. 2009). Then, SSUmCf and LSUmBr were applied as nested primers. The final concentration of the PCR reaction mix contained 0.02 U μL^{-1} Phusion polymerase, 1X Phusion HF Buffer with 1.5 mM $MgCl_2$, 200 μM of each dNTP (Thermofisher, USA), and 0.5 μM of each primer, The thermal cycling was done in the S1000 Thermal Cycler™ (BIORAD, Hercules, CA, United States) in a volume of 25 μL, with the following conditions for the first PCR: initial denaturation at 98 °C for 3 min, 35 cycles at 98 °C for 10 s, primer annealing at 60 °C for 30 s, extension at 72 °C for 1 m, and a final extension at 72 °C for 10 min. The same conditions were used for the nested PCR primers except that the annealing temperature that was 63 °C and only 30 cycles. Negative and positive controls were used in each PCR cycle. The PCR products were loaded on 2% agarose gels (UltraPure Agarose, Thermofisher, USA) with 1X Tris–Borate-EDTA (TBE) at 100 V and stained with 0.5 μg mL^{-1} ethidium bromide. The pool of the three amplicons of DNA per each replicate was ligated into the pGem®-T Easy vector (Promega, Germany) and the production of ligation were transformed by XL10-Gold® Ultracompetent *Escherichia coli* cells (Agilent Technologies, Italy). On average 56 ± 2 recombinant clones per amplicon library (i.e., per replicate) were positive for the ca. 1500-bp-long fragment in var. Pisanello at 2nd harvest. For. Var. Rio Grande on average 26 ± 1 and 57 ± 4 recombinant clones per amplicon library (i.e., per replicate) were positive at transplanting and 2nd harvest, respectively. Cloning efficiency was on average 71%. Amplification was done using the GoTaq DNA Polymerase (Promega, Germany) (5 U μL^{-1}) and primers pair SP6 and T7. PCR amplicons of the clones were visualized on agarose gels using 2% ultrapure agarose (VWR), 1X Tris–Borate-EDTA (TBE) and ethidium bromide (0.5 μg mL^{-1}). Positive clones were converted to plasmids using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich, USA). Sequencing reactions were set up with the vector primers SP6 and T7 using on an ABI 3730XL sequencing machine at the Eurofins Genomics (Germany).

Statistical and bioinformatic analyses

One-way analysis of variance (ANOVA) was performed on fruit quality parameters. Orthogonal contrasts were used to test differences between means: + M (all four isolates) *vs.* − M (mock inoculated controls) (1st comparison), Gigasporaceae *vs.* Glomeraceae (2nd comparison: inter-family diversity), between the two isolates of Gigasporaceae (3rd comparison: intra-family diversity), and between the two isolates of Glomeraceae (4th comparison: intra-family diversity). The effect of the transformation process (tomato fruits *vs.* sauce) on the fruit quality parameters of both tomato varieties was tested by one-way ANOVA, using the inoculation treatment as covariate. The data were ln- or arcsine-transformed when needed to fulfil the assumptions of ANOVA. Means and standard errors given in figures are for untransformed data. All analyses were performed using the software package on SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

The Glomeromycota affiliation of the sequences was verified in similarity searches using the Basic Local Alignment Search Tool (BLASTn) in the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al. 1997). No chimeric sequences were detected among the 765, 211 and 788 newly generated AM fungal sequences (ca. 1500 bp) obtained from var. Pisanello at 2nd harvest and from var. Rio Grande at transplanting and 2nd harvest, respectively. A total number of 67 ± 1, 185 ± 7, and 61 ± 2 non-Glomeromycota sequences were obtained from var. Pisanello at 2nd harvest and from var. Rio Grande at transplanting and 2nd harvest, respectively. The AM fungal sequences obtained from roots sampled at the 2nd harvest of var. Pisanello were aligned according to Krüger et al. (2012) (http://www.amf-phylogeny.com) that allows a robust resolution and avoids artificial clustering resulting from misalignment or from convergent characters resulting from mutational saturation in the highly variable regions. The AM fungal sequences obtained from roots sampled at transplanting and at the 2nd harvest of var. Rio Grande were aligned using the same method. Three phylogenetic trees were built using the maximum likelihood (ML) analysis in MEGA11 by 100 iterations for the bootstrap method, Tamura-Nei model, and using all sites (Tamura et al. 2021). The phylograms were drawn by MEGA 11 and edited by Adobe Illustrator 2021. All representative newly generated sequences are deposited in NCBI Sequence Read (SRA) database as SUB13362899, SUB13324118, and SUB13375274.

The relative abundance of the AMF in each sample was calculated based on the ratio between the number of sequences affiliated to each phylotype and the total number of sequences obtained from the clone library. Total number of AM fungal phylotypes (Richness, S) and diversity indexes (Shannon index, H' and Simpson index, λ) were calculated per each sample. The H' and λ were calculated as follows: $H' = \mathrm{SUM}(Pi*\mathrm{Log10}(Pi))$ and $1-\lambda' = 1-\mathrm{SUM}(Ni*(Ni-1)/(N*(N/1))$. In the formulas, Pi is the proportion of individuals belonging to the ith phylotype, Ni is the number of individuals to the ith phylotype, and N is the total number of the individuals of all phylotypes. One-way analysis of variance (ANOVA) was performed on the AM fungal phylotype richness and diversity indexes, and the differences between means were determined using orthogonal contrasts, following the pair-wise contrasts as defined above. At the 2nd harvest for Pisanello and at transplanting and 2nd harvest for Rio Grande, the cluster/similarity profile (SIMPROF) analysis was used to group the different samples into clusters based on their similarity/homogeneity of AM fungal intraradical communities (relative abundances of phylotypes) and to group the different AM fungal phylotypes based on their similarity of occurrence. Relative AM fungal abundance data were initially $\log(X+1)$ transformed (Clarke and Warwick 2001) and the Bray–Curtis similarity was calculated. The SIMPROF cluster analysis was performed to objectively define the groups within the dendrogram. Moreover, for Pisanello and Rio Grande, the relative abundances of the AM fungal phylotypes found at 2nd harvest and at transplanting and 2nd harvest, respectively, were represented by a shade plot.

To understand in the two tomato varieties the functional relationship between AM fungal colonization traits (i.e., arbuscules, vesicles, AM fungal root colonization; data available in Arcidiacono et al. 2023) and fruit quality parameters (TPC, ORAC, and lycopene), and between AM fungal community (relative abundances of AM fungal phylotypes) and fruit quality parameters, and to understand which were the main responsible traits/phylotypes, a multivariate statistical approach was applied utilising data of samples collected at 2nd harvest. A principal component analysis (PCA) was performed on the Euclidean distance matrix of the AM fungal colonization traits that were square root-transformed data and a PCA was performed on the Euclidean distance matrix of the tomato quality parameters that were square root-transformed and standardized (Abdi and Williams 2010). A non-metric multidimensional scaling analysis (nMDS) was performed on the Bray–Curtis similarity matrix calculated on the fourth-root AM fungal relative abundances of phylotypes in inoculated and not-inoculated tomato varieties (Kruskal 1964). The relationship between AM fungal colonization traits and tomato quality parameters, and between AM fungal root community and tomato quality parameters was determined by a RELATE analysis that allows to determine the strength of the correlation between two matrices in rank-order patterns of dissimilarity (Clarke and Warwick 2001). The analysis was based on Spearman rank and 999 permutations with ρ equal to 1 representing

perfect relationship. To find the best descriptor of the relationships, the BEST analysis, based on BioEnv methods (all combinations), Spearman 529 rank and 999 permutations, was applied (Clarke et al. 2008). Finally, the distance-based linear method (DistLM) analysis, using a stepwise selection and the Akaike's information criterion (AICc), was applied to measure the significance and the variance explained by the best descriptor/s (Knorr et al. 2000), and the distance-based redundancy analysis was used to plot the first and second axes of the DistLM (Legendre and Anderson 1999).

Results

Total phenols in fruits

In 2019, at both 1st and 2nd harvests, AM fungal inoculation increased total phenols concentration in fruits of var. Pisanello (Fig. 1a, c; Tables S2 and S3). The increase was about 31% at 1st harvest and 45% at 2nd harvest. Similarly, in 2020, at both harvests, AM fungal inoculation increased TPC in fruits of var. Rio Grande (Fig. 1e, h), with 78 and 35% increases at 1st and 2nd harvests, respectively. A significant inter-family variability was found only in var. Rio Grande (Fig. 1f, i). At first harvest, fruits of Rio Grande inoculated with Gigasporaceae had a higher TPC than those of plants inoculated with Glomeraceae (+ 10%), whereas at 2nd harvest, the opposite behavior was observed (− 15%). In var. Pisanello, fruits of plants inoculated with *G. gigantea* had higher values of TPC than those of plants inoculated with *S. pellucida*, at both harvests, by 11 and 15%, respectively (Fig. 1b, d). By contrast, fruits of plants of var. Rio Grande inoculated with *G. gigantea*, at the first harvest, showed values of TPC 15% lower than those of plants inoculated with *S. pellucida* (Fig. 1g).

Antioxidant activity in fruits

In 2019, at first harvest, inoculation with *G. gigantea* increased by 5% the antioxidant activity (ORAC) of fruits of var. Pisanello when compared with *S. pellucida* (Fig. 2a, Tables S2 and S3). Accordingly, at 2nd harvest, fruits of plants of var. Rio Grande inoculated with *G. gigantea* showed twofold higher values of ORAC compared with those of plants inoculated with *S. pellucida* (Fig. 2f). In 2020, at both harvests, AM fungal inoculation increased ORAC in fruits of var. Rio Grande (Fig. 2b,d). The increase was about 122% at 1st harvest and 48% at 2nd harvest. In addition, in var. Rio Grande, a consistent inter-family variability was recorded at both harvests: higher values of ORAC

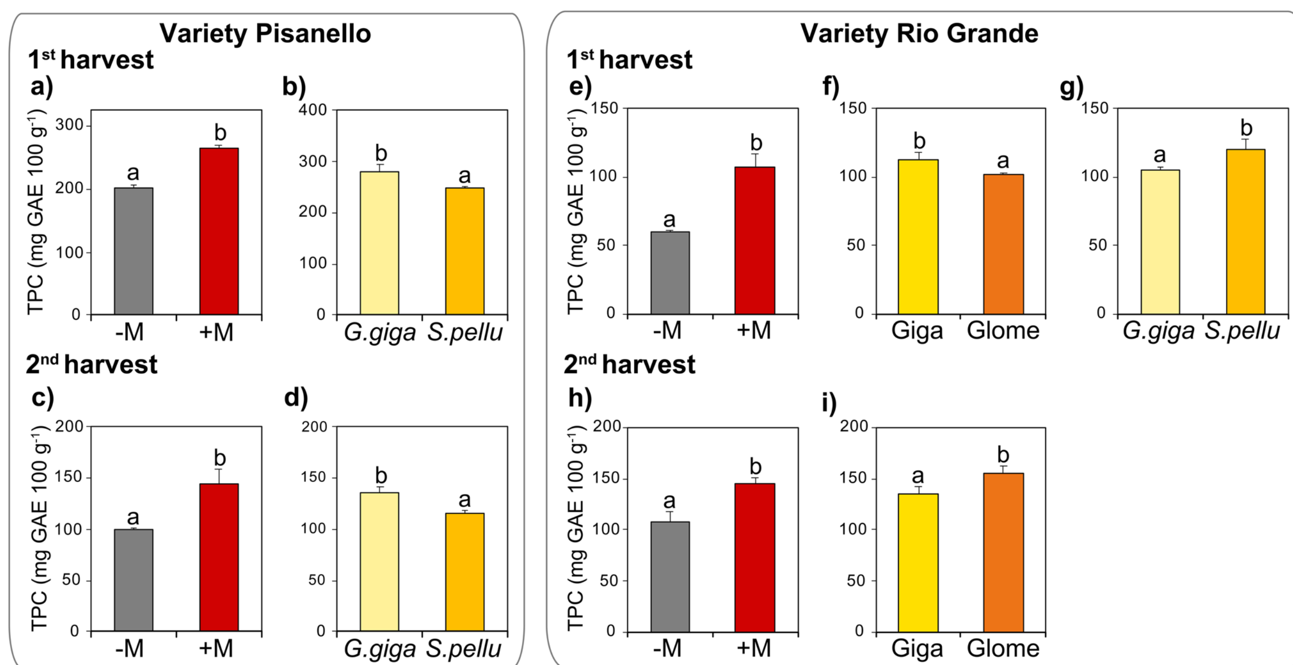


Fig. 1 Total phenolic content (TPC) (mg of gallic acid equivalent, GAE, per 100 g of dry weight) in fruits of *Solanum lycopersicum* L. var. Pisanello at 1st and 2nd harvests: -M (mock inoculation, control) *vs.* + M (AM fungal inoculation) ($n=3$ and $n=12$, respectively) (**a**, **c**); *Gigaspora gigantea* (*G.giga*) vs. *Scutellospora pellucida* (*S. pellu*) ($n=3$) (**b**, **d**). TPC in fruits of *Solanum lycopersicum* L. var. Rio Grande at 1st and 2nd harvests: − M *vs.* + M ($n=3$ and $n=12$, respectively) (**e**, **h**); Gigasporaceae (Giga) *vs.* Glomeraceae (Glome) ($n=6$) (**f**, **i**). TPC in fruits of var. Rio Grande at first harvest: *G.giga vs. S.pellu* ($n=3$) (**g**). Figure reports only the significant results among all the tested linear orthogonal contrasts (Table S2). Values are mean ± SE of three replicate plots per treatment

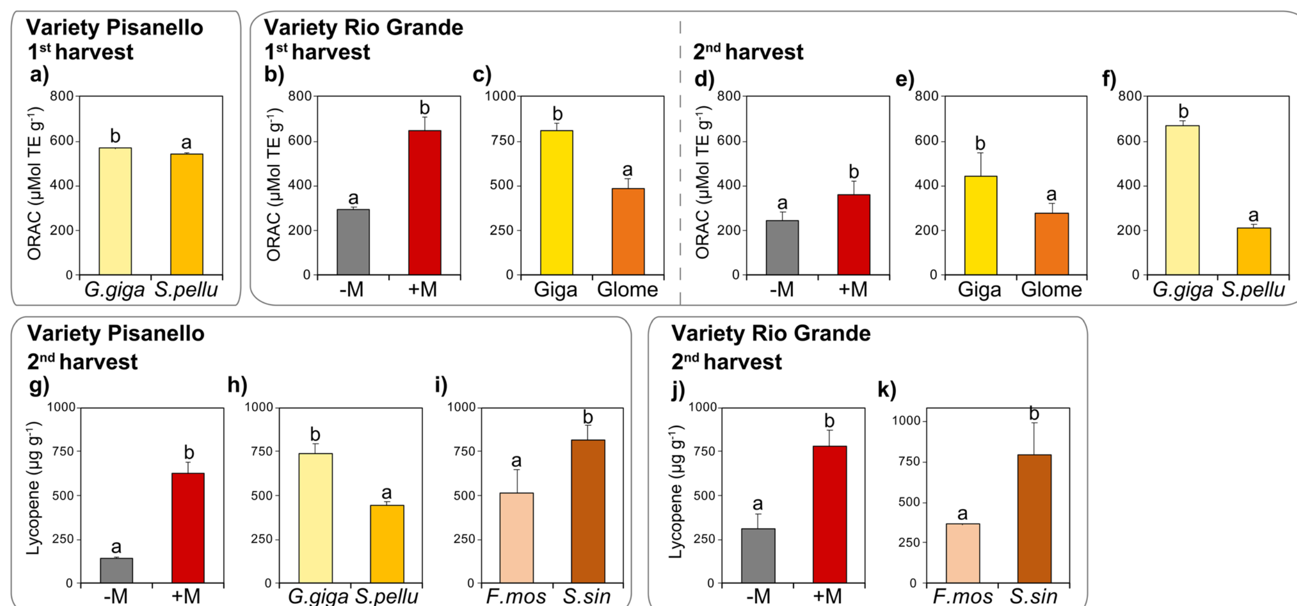


Fig. 2 Antioxidant activity (oxygen radical absorbance capacity, ORAC) (μMol Trolox equivalent antioxidant capacity per g of dry weight) in fruits of *Solanum lycopersicum* L. var. Pisanello at first harvest: *Gigaspora gigantea* (*G.giga*) *vs.* *Scutellospora pellucida* (*S. pellu*) ($n=3$) (**a**). ORAC in fruits of var. Rio Grande at 1st and 2nd harvests: − M *vs.* + M ($n=3$ and $n=12$, respectively) (**b**, **d**); Gigasporaceae (Giga) *vs.* Glomeraceae (Glome) ($n=6$) (**c**, **e**). TPC in fruits of var. Rio Grande at 2nd harvest: *G.giga vs. S.pellu* ($n=3$) (**f**). Lycopene (μg per g of dry weight) in fruits of *Solanum lycopersicum* L. var. Pisanello and Rio Grande at 2nd harvest: − M *vs.* + M ($n=3$ and $n=12$, respectively) (**g**, **j**); *Funneliformis mosseae* (*F.mos*) *vs.* *Sclerocystis sinuosa* (*S.sin*) ($n=3$) (**i**, **k**). Lycopene in fruits of var. Pisanello at 2nd harvest: *G.giga vs. S.pellu* ($n=3$) (**h**). Figure reports only the significant results among all the tested linear orthogonal contrasts (Table S2). Values are mean ± SE of three replicate plots per treatment

where recorded in fruits of plants inoculated with Gigasporaceae compared with Glomeraceae, by 66 and 60% at 1st and 2nd harvests, respectively (Fig. 2c, e).

Lycopene in fruits

AM fungal inoculation greatly improved the lycopene concentration of tomato fruits of both Pisanello and Rio Grande varieties at the 2nd harvest (Fig. 2g, j; Tables S2 and S3). The increase was 344% in Pisanello and 150% in Rio Grande. Moreover, fruits of plants of var. Pisanello inoculated with *G. gigantea* showed higher lycopene values (+ 66%) when compared to fruits of plants inoculated with *S. pellucida* (Fig. 2h). Finally, a consistent intra-family variability was detected within Glomeraceae in both tomato varieties, with fruits of plants inoculated with *S. sinuosa* having higher values of lycopene respect to fruits of plants inoculated with *F. mosseae* (Fig. 2i, k). The increase was 59% in Pisanello and 118% in Rio Grande.

Tomato sauce quality parameters

AM fungal inoculation did not affect TCP, ORAC, and lycopene concentration in tomato sauce of both varieties, while differences in quality parameters at inter- and intra-family level were detected (Fig. 3; Tables S4 and S5). In var. Pisanello, lycopene was promoted in the sauce obtained from fruits of plants inoculated with Gigasporaceae in comparison with Glomeraceae (Fig. 3c; Table S4). Similarly, in var. Rio Grande, TPC and ORAC were higher in the tomato sauce from the inoculation with Gigasporaceae than in that one obtained from the inoculation with Glomeraceae (Fig. 3e,h). Conversely, for lycopene an opposite behavior was observed (Fig. 3j). In addition, an intra-family variability was found in Gigasporaceae for lycopene and TPC values of the tomato sauce of var. Pisanello and var. Rio Grande, respectively. Indeed, both quality parameters were increased by the inoculation with *S. pellucida* (Fig. 3d, f). Conversely, in var. Rio Grande, the ORAC was increased by the inoculation with *G. gigantea* (Fig. 3j). An intra-family variability was also found in Glomeraceae. Indeed, in var. Pisanello, the inoculation with *S. sinuosa* increased the content of TPC and ORAC in tomato sauce (Fig. 3a, b), whereas in var. Rio Grande, TPC content was increased by the inoculation with *F. mosseae* (Fig. 3g).

Moreover, the transformation process decreased quality parameters, such as TPC and ORAC in the sauce of var. Pisanello and TPC (Fig. 4a, b; Table S6) and lycopene in the sauce of var. Rio Grande (Fig. 4d; Table S6). By contrast, lycopene in the sauce of var. Pisanello as well as ORAC in

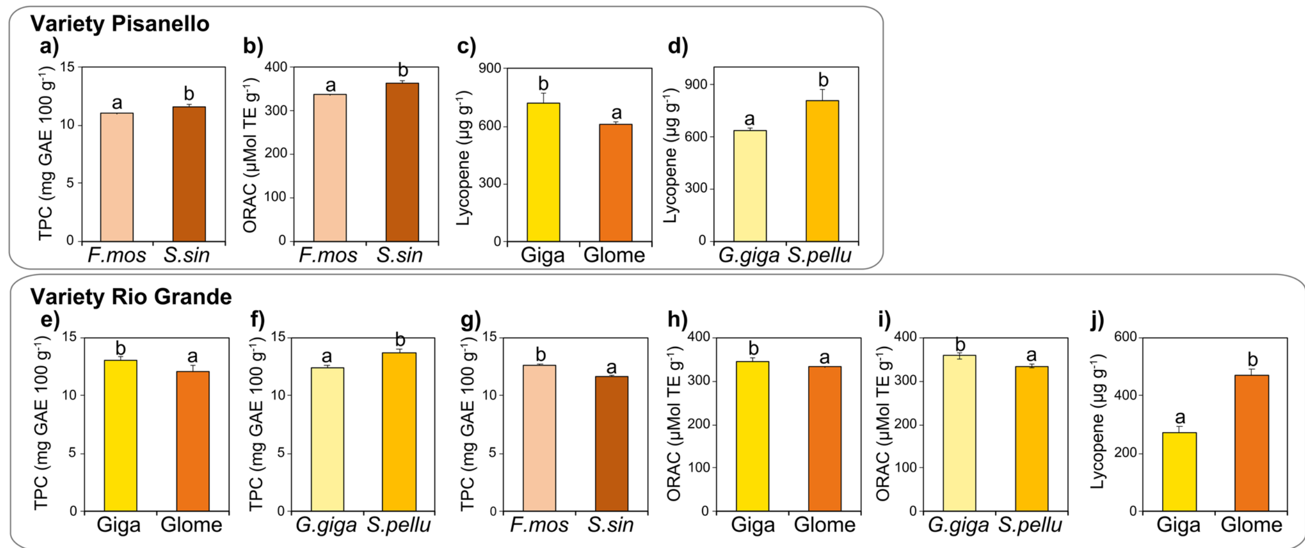


Fig. 3 Total phenolic content (TPC) (mg of gallic acid equivalent, GAE, per 100 g of dry weight) in the tomato sauce obtained from fruits of *Solanum lycopersicum* L. var. Pisanello: *Funneliformis mosseae* (*F.mos*) *vs. Sclerocystis sinuosa* (*S.sin*) ($n=3$) (**a**). TPC in the sauce of var. Rio Grande: Gigasporaceae (Giga) *vs.* Glomeraceae (Glome) ($n=6$) (**e**); *Gigaspora gigantea* (*G.giga*) *vs. Scutellospora pellucida* (*S.pellu*) ($n=3$) (**f**); *F.mos vs. S.sin* ($n=3$) (**g**). Antioxidant activity (oxygen radical absorbance capacity, ORAC) (μMol Trolox equivalent antioxidant capacity per g of dry weight) in the sauce of var. Pisanello: *F.mos vs S.sin* ($n=3$) (**b**). ORAC in the sauce of var. Rio Grande: Giga *vs.* Glome ($n=6$) (**h**); *G.giga vs. S.pellu* ($n=3$) (**i**). Lycopene (μg per g of dry weight) in the sauce of var. Pisanello: Giga *vs* Glome ($n=6$) (**c**); *G.giga vs. S.pellu* ($n=3$) (**d**). Lycopene (μg per g of dry weight) in the sauce of var. Rio Grande: Giga *vs.* Glome ($n=6$) (**j**). Figure reports only the significant results among all the tested linear orthogonal contrasts (Table S3). Values are mean and SE of three replicate plots per treatment

the sauce of var. Rio Grande were not significantly affected by the transformation process (Fig. 4c,e; Table S6).

Diversity of AMF in roots of tomato plants

Using the small subunit-internal transcribed spacer-large subunit (SSU-ITS-LSU) rDNA sequence, foreign-inoculated strains of *G. gigantea*, *S. pellucida*, *F. mosseae*, and *S. sinuosa* were retrieved at 2nd harvest within the roots of tomato var. Pisanello (Figs. 5, S1) as well as at transplanting and 2nd harvest within the roots of tomato var. Rio Grande (Figs. 6 and 7, S2 and S3). In var. Pisanello, the phylogenetic ML tree, built aligning 765 newly generated AM fungal sequences with reference sequences of Krüger et al. (2012), allowed to discriminate a total of 11 AM fungal phylotypes (Figs. 5, S1). The AM fungal richness (S) in the inoculated roots of cv. Pisanello ranged from three to seven in *G. gigantea* and *F. mosseae*, respectively, while within the mock-inoculated roots (−M) six AM fungal phylotypes were retrieved (Table S7). Statistically significant differences were found in S, Shannon index (H') and Simpson index (λ) between + M and − M, as well as between and within AM fungal families (Tables S7 and S8). Overall, inoculation did not determine large variation in AM fungal diversity (+ M *vs.* − M) (Table S7). Inoculants belonging to Glomeraceae did not modify AM fungal diversity, whereas those belonging to Gigasporaceae generally reduced diversity. Within the family Glomeraceae, *F. mosseae* showed a diversity pattern similar to − M. Looking at root community (Fig. S5), 45% and 29% of the AM fungal sequences retrieved in *G. gigantea* and *S. pellucida* inoculated plants were affiliated to *G. gigantea* and *S. pellucida*. Similarly, in roots inoculated with *F. mosseae* and *S. sinuosa*, 36% and 22% of the retrieved AM fungal sequences were affiliated to *F. mosseae* and *S. sinuosa*. By contrast, in mock-inoculated roots, high relative abundances of *Funneliformis* sp., *Rhizophagus irregularis*, *Entrophospora claroidea*, and *Racocetra fulgida* were retrieved, while no AM fungal sequences affiliated to *G. gigantea*, *S. pellucida, F. mosseae*, and *S. sinuosa* were found (Fig. S5). Consistently with the diversity patterns, the cluster analysis allowed to identify five SIMPROF supported clusters that were consistent with the AM fungal inoculation levels (Fig. 7a). However, root samples of plants inoculated with *F. mosseae* and *S. sinuosa* (Glomeraceae) showed an AM fungal community similar to the mock-inoculated control (ca. 70% and 60% similarity, respectively), whereas those inoculated with *G. gigaspora* and *S. pellucida* (Gigasporaceae) clustered together and far apart (ca. 30% similarity). The AM fungal phylotypes retrieved within the roots of var. Pisanello clustered in four different groups, irrespective

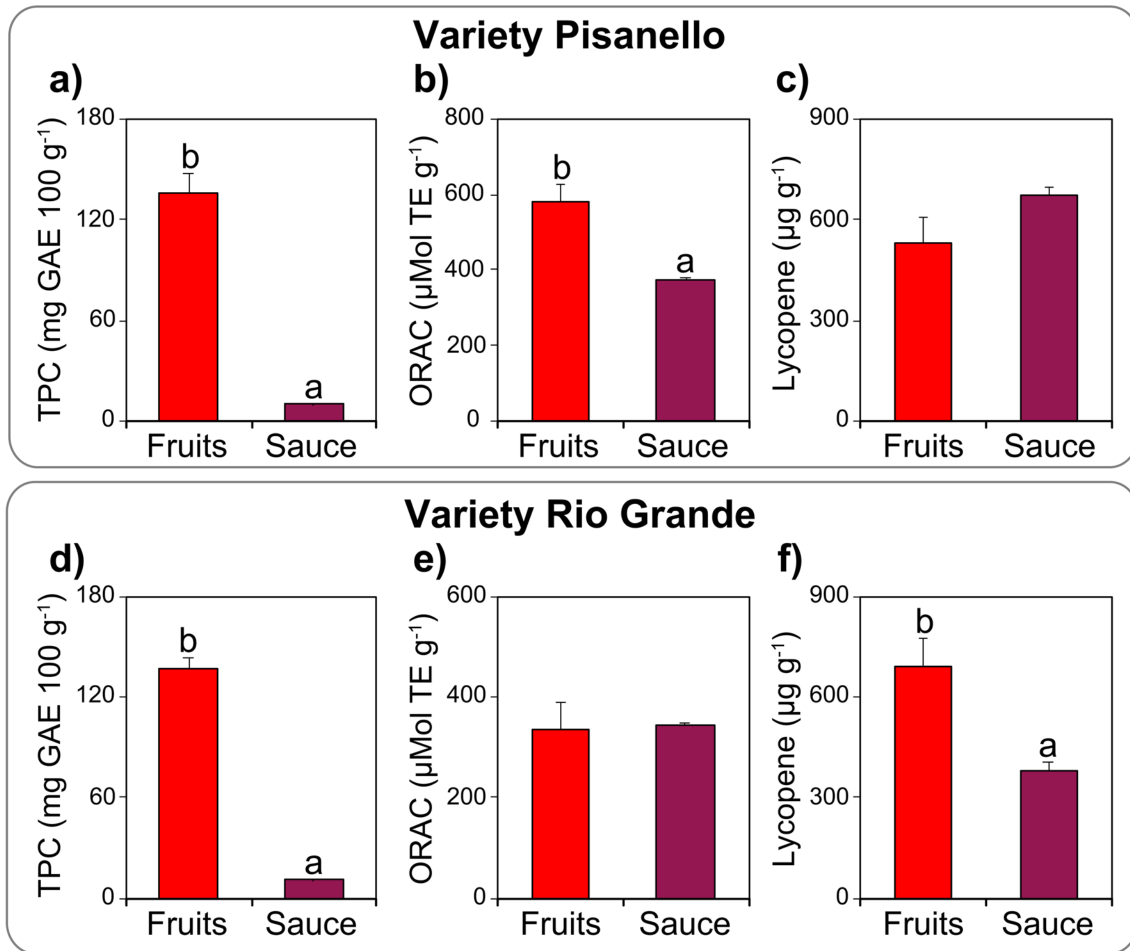


Fig. 4 Effect of the transformation process (tomato fruits *vs.* sauce) on the quality parameters of tomato (*Solanum lycopersicum* L.) var. Pisanello and var. Rio Grande: total phenolic content (TPC) (mg of gallic acid equivalent, GAE, per 100 g of dry weight) (**a**, **d**); antioxidant activity (oxygen radical absorbance capacity, ORAC) (µMol Trolox equivalent antioxidant capacity per g of dry weight) (**b**, **e**); lycopene (µg per g of dry weight) (**c**, **f**). Values are mean ± SE of 15 replicates per treatment

to inoculation (Fig. 7a). As example, *G. gigantea*, *R. irregularis*, and *S. pellucida* clustered together.

At the transplanting of tomato var. Rio Grande, the phylogenetic ML tree, built aligning 211 newly generated AM fungal sequences with reference sequences of Krüger et al. (2012), allowed to successfully verify the unique presence of the AM fungal inoculants within the roots sampled in each corresponding treatment (Figs. S2 and S3). Moreover, no AM fungal sequences were retrieved in roots of mock-inoculated controls. At 2nd harvest, the phylogenetic ML tree, built aligning 788 newly generated AM fungal sequences with reference sequences of Krüger et al. (2012), allowed to discriminate a total of 15 AM fungal phylotypes (Figs. 6, S4). The AM fungal richness (S) in the inoculated roots of var. Rio Grande ranged from three to eleven in *G. gigantea* and *F. mosseae*, respectively, while within the mock-inoculated roots (−M) seven AM fungal phylotypes were retrieved (Table S7). Statistically significant differences were found in S, Shannon index (H') and Simpson index (λ) between + M and − M, as well as between and within AM fungal families (Tables S7 and S8). Overall, inoculation did not determine large variation in AM fungal diversity (+ M *vs* -M) (Table S7). Inoculants belonging to Glomeraceae increased AM fungal diversity, whereas those belonging to Gigasporaceae generally reduced diversity. Within the family Glomeraceae, *F. mosseae* promoted the highest diversity. Looking at root community (Fig. S5), 45% and 49% of the AM fungal sequences retrieved in *G. gigantea* and *S. pellucida* inoculated plants were affiliated to *G. gigantea* and *S. pellucida*. Similarly, in roots inoculated with *F. mosseae* and *S. sinuosa*, 39% and 29% of the retrieved AM fungal sequences were affiliated to *F. mosseae* and *S. sinuosa*. By contrast, in mock-inoculated roots, high relative abundances of *Funneliformis* sp., *Rhizophagus* sp., *R. irregularis*, and *Funneliformis coronatus* were retrieved, while no AM fungal

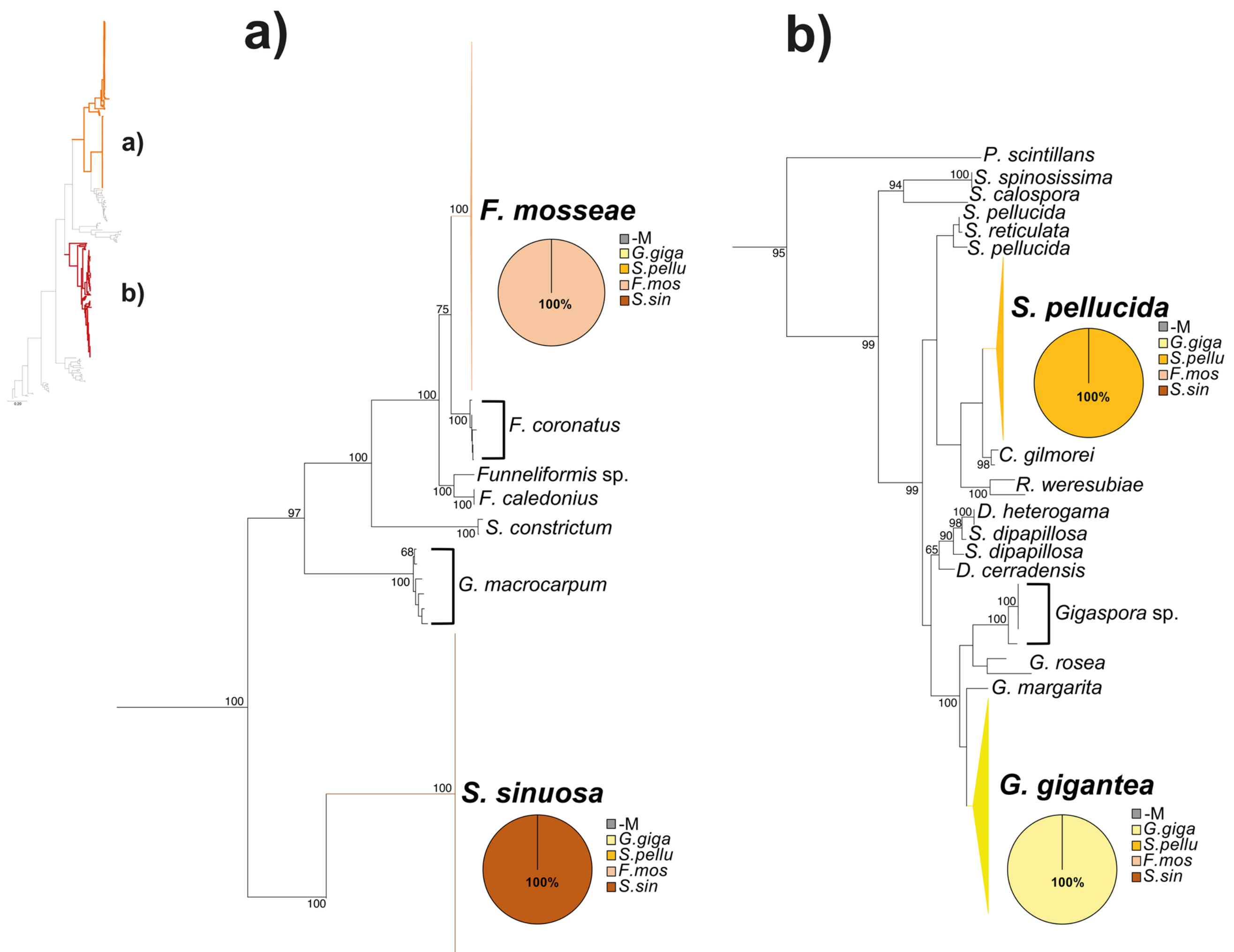
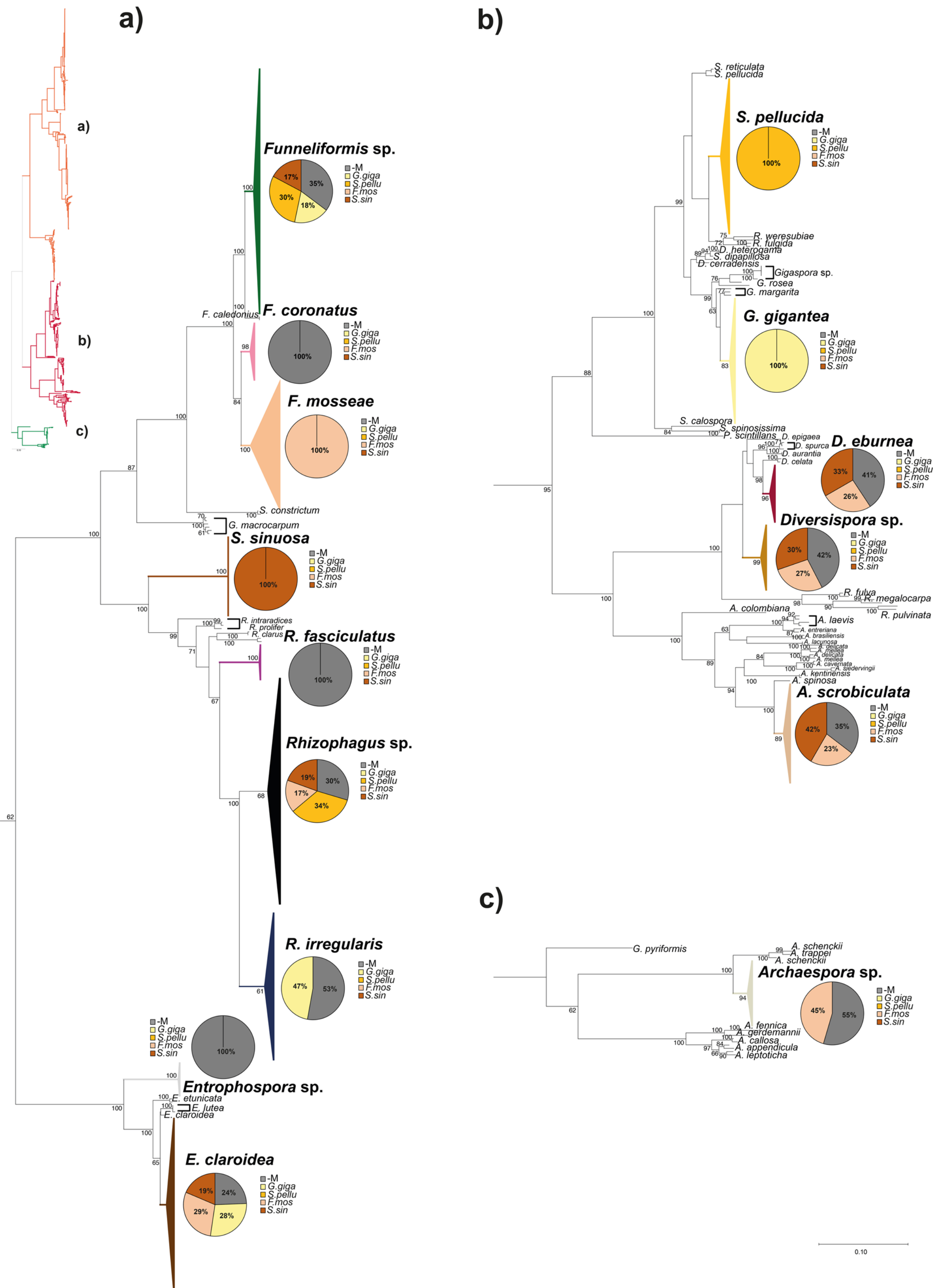


Fig. 5 Collapsed maximum likelihood (ML) tree of sequences of AMF (Glomeromycota) retrieved at 2nd harvest within the roots of tomato (*Solanum lycopersicum* L.) var. Pisanello inoculated with AMF at nursery and mock inoculated (control, – M). Inocula were: *Gigaspora gigantea* (*G.giga*) and *Scutellospora pellucida* (*S.pellu*) belonging to Gigasporaceae, and *Funneliformis mosseae* (*F.mos*) and *Sclerocystis sinuosa* (*S.sin*) belonging to Glomeraceae. The ML tree is based on sequences obtained from the amplification of part of the SSU, the complete ITS region, and ca. 0.8 kb of the LSU rRNA gene (ca. 1500-bp-long fragment) (Krüger et al 2009). The tree is composed by 765 newly generated sequences belonging to 11 phylotypes, plus 161 reference sequences from NCBI (see Fig. S1 for the whole ML tree). Bootstrap values (based on 1000 replicates) are shown at the nodes. The scale bar indicates substitutions per site. Portions of the ML tree are shown in (**a**) and (**b**). Collapsed phylotypes are shown by coloured branches and triangles. Pie charts represent the relative abundances of each phylotypes. The sequences of *Paraglomus occultum* IA702 was used as an outgroup. The newly generated sequences are highlighted in boldface, see collapsed branches

sequences affiliated to *G. gigantea*, *S. pellucida, F. mosseae*, and *S. sinuosa* were found (Fig. S5). Consistently with the diversity patterns, the cluster analysis allowed to identify five SIMPROF supported clusters that were consistent with the AM fungal inoculation levels (Fig. 7b). However, root samples of plants inoculated with *S. sinuosa* and *F. mosseae* (Glomeraceae) showed an AM fungal community similar to the mock-inoculated control (ca. 70%), whereas those inoculated with *G. gigaspora* and *S. pellucida* (Gigasporaceae) clustered separately and far apart (ca. 30% similarity). The AM fungal phylotypes retrieved within the roots of var. Rio Grande clustered in four different groups, irrespective to inoculation (Fig. 7b). As example, *G. gigantea* and *R. irregularis* clustered together.

Functional relationship between AM fungal traits and tomato quality parameters

Mock-inoculated controls were well separated from the inoculated ones through the PCA along the first axis, based on the AM fungal colonization parameters assessed in the roots of tomato var. Pisanello (Fig. 8a). Moreover, the PCA based on the corresponding tomato quality parameters, well separated the non-inoculated plots from the inoculated



ones along the first axis (Fig. 8b). Overall, arbuscules and AM fungal colonization were positively and strongly correlated with lycopene, while vesicles with ORAC and TPC. The significance of the relationship between AM fungal colonization traits and tomato quality parameters was supported by the RELATE analysis, for which the ρ was equal to 0.30 ($P = 0.028$) (Fig. S6a). BEST analysis allowed to highlight that arbuscules and vesicles were good predictors

◂**Fig. 6** Collapsed maximum likelihood (ML) tree of sequences of AMF (Glomeromycota) retrieved at 2nd harvest within the roots of tomato (*Solanum lycopersicum* L.) var. Rio Grande inoculated with AMF at nursery and mock inoculated (control, – M). Inocula were *Gigaspora gigantea* (*G.giga*) and *Scutellospora pellucida* (*S.pellu*) belonging to Gigasporaceae, and *Funneliformis mosseae* (*F.mos*) and *Sclerocystis sinuosa* (*S.sin*) belonging to Glomeraceae. The ML tree is based on sequences obtained from the amplification of part of the SSU, the complete ITS region, and ca. 0.8 kb of the LSU rRNA gene (ca. 1500-bp-long fragment) (Krüger et al. 2009). The tree is composed by 788 newly generated sequences belonging to 15 phylotypes, plus 162 reference sequences from NCBI (see Fig. S4 for the whole ML tree). Bootstrap values (based on 1000 replicates) are shown at the nodes. The scale bar indicates substitutions per site. Portions of the ML tree are shown in (**a**), (**b**) and (**c**). Collapsed phylotypes are shown by coloured branches and triangles. Pie charts represent the relative abundances of each phylotypes. The sequences of *Paraglomus occultum* IA702 was used as an outgroup. The newly generated sequences are highlighted in boldface, see collapsed branches

(correlation:0.343; data not shown) (Table S9), while DistLM analysis supported the main role played by arbuscules in determining the pattern of quality parameters in tomato (Fig. 8e; Table S9). Similarly, in var. Rio Grande, the PCA based on the AM fungal colonization parameters, and that one based on tomato quality parameters well separated the non-inoculated plots from the inoculated ones along the first axis (Fig. 8c, d). Overall, arbuscules and AM fungal colonization were positively and strongly correlated with lycopene, while vesicles with ORAC. The significance of the relationship between AM fungal colonization traits and tomato quality parameters was supported by the RELATE analysis, for which the ρ was equal to 0.21 ($P = 0.043$) (Fig. S6b). BEST analysis allowed to highlight that arbuscules were good predictors (correlation:0.256; data not shown) (Table S9) and DistLM analysis supported their main role in determining the pattern of quality parameters in tomato (Fig. 8f; Table S9).

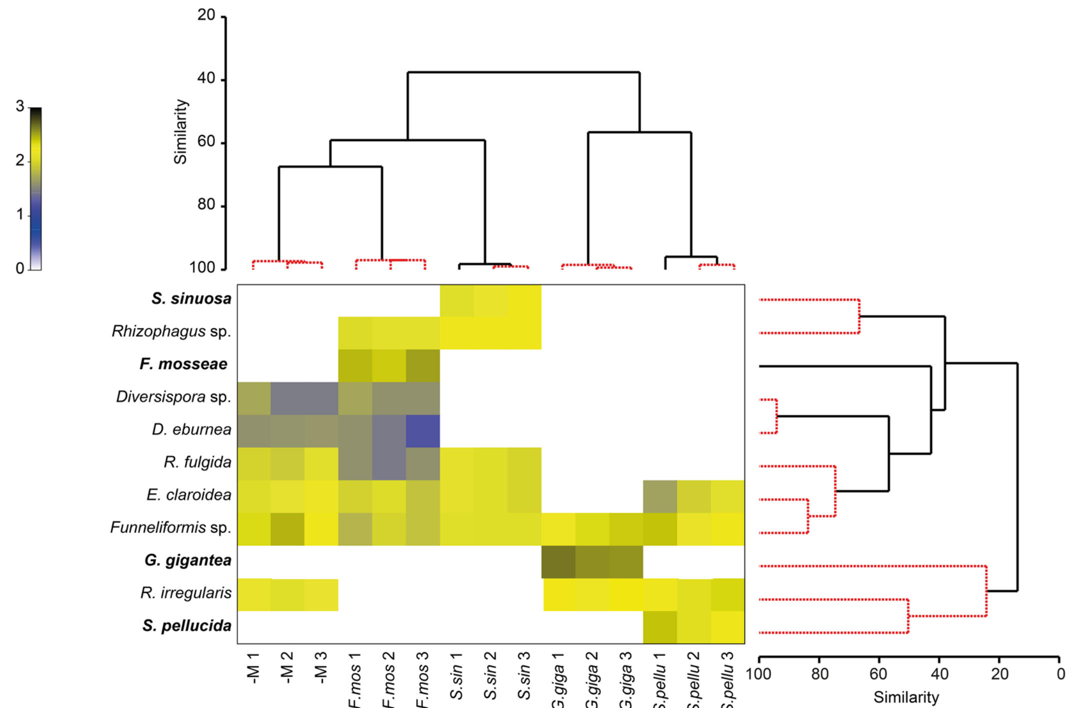
In var. Pisanello and var. Rio Grande, nMDS plots, representing AM fungal communities within roots, showed that all treatments were well separated among each other (Fig. 9a, b). *Funneliformis mosseae* and *S. sinuosa* inoculated plants of both varieties showed root AM fungal communities similar to mock-inoculated control. Moreover, in var. Pisanello, Glomeraceae inoculated plants and control showed root AM fungal communities strongly different from those retrieved in roots of *G. gigantea* and *S. pellucida* inoculated plants (Fig. 9a). A similar pattern was observed in var. Rio Grande, although *G. gigantea* and *S. pellucida* inoculated plants had a community also distinct between each other (Fig. 9b). Looking at the RELATE analysis, no significant relationship was found between root AM fungal community and quality parameters in var. Pisanello (Rho:0.019; $P = 0.376$) (Fig. S6c). By contrast, in var. Rio Grande, a significant relationship was supported by the RELATE analysis, for which the ρ was equal to 0.49 ($P = 0.001$) (Fig. S6d). BEST analysis allowed to highlight that *Archeospora* sp., *G. gigantea*, *Rhizophagus fasciculatus*, and *Rhizophagus* sp. were good predictors (correlation:0.735; data not shown) (Table S9), and DistLM analysis supported the main role played by *Archeospora* sp., *R. fasciculatus*, and *Rhizophagus* sp. in determining the pattern of quality parameters in tomato (Fig. 9c; Table S9).

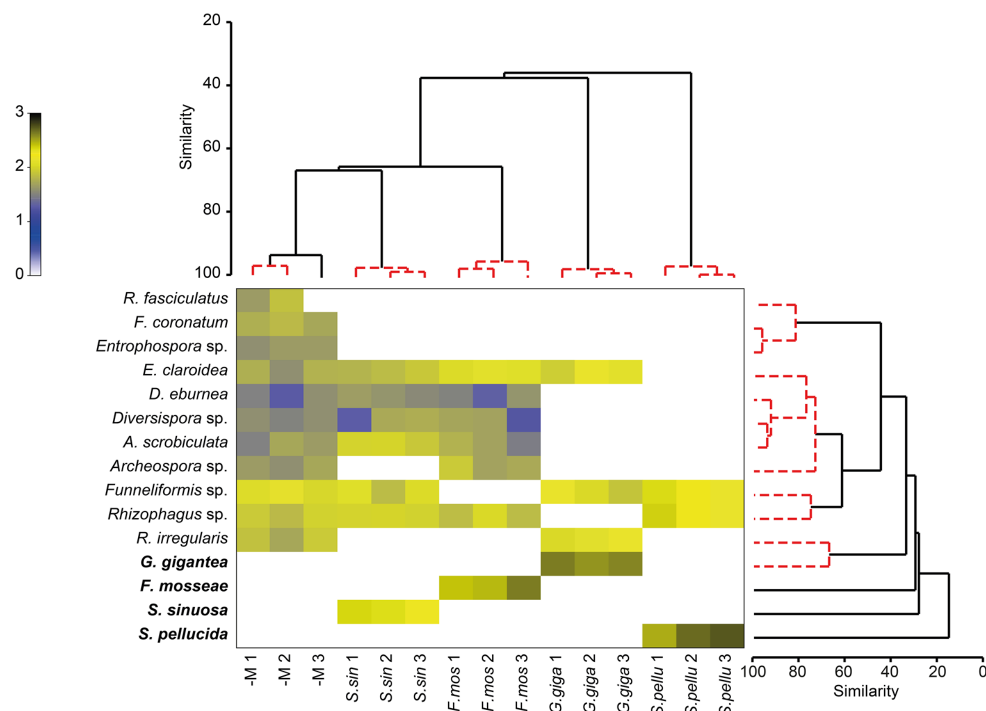
Discussion

Health-related compounds in fruits

In a previous work, we demonstrated the positive effect of AM fungal inoculation on yield and nutrient concentration in fruits of the tomato varieties Pisanello and Rio Grande (Arcidiacono et al. 2023). Overall, inoculants with members of Gigasporaceae were more effective in increasing the concentration of nutrients in fruits compared to Glomeraceae. However, within Gigasporaceae, the positive effect on nutrients was similar between *G. gigantea* and *S. pellucida*, whereas, within Glomeraceae, *F. mosseae* determined larger increases of several nutrients compared with *S. sinuosa*. In this work, we confirmed the positive effect of AM fungal inoculation on total phenols, antioxidant activity and lycopene in fruits. Our findings on TPC and lycopene are in accordance with previous works on several varieties of tomato (Carillo et al. 2020; Copetta et al. 2011; Giovannetti et al. 2012; Hart et al. 2015; Pasković et al. 2021; Ulrichs et al. 2008). Conversely, our results on antioxidant activity in fruits partially disagree with the results of Giovannetti et al. (2012) and Hart et al. (2015). Indeed, they reported that inoculation did not affect this parameter, whereas in our work antioxidant activity was not modified in var. Pisanello and increased in var. Rio Grande. It is also important to highlight that in the previous works the authors utilized assays based on 2,2′ azino-bis-3-ethylbenzthiazoline-6-sulfonic acid based (ABTS) and on 2,2-dipheniyl-1-picrylhydrazyl (DPHH) assays, which are less sensitive than the ORAC assay that is based on fluoresceine. It is interesting to notice that the increase in lycopene concentration showed in our work (150% and 344% in var Pisanello and var. Rio Grande, respectively) is much higher compared to the findings of Giovannetti et al. (2012) and Carillo et al. (2020), recording 19% and 47% increases, respectively. However, these authors measured lycopene on fresh basis, whereas our measures were performed on lyopilized samples.

The tested AM fungal isolates produced host benefits measured as health-related compounds variable and not fully consistent with our original hypothesis of a higher accumulation under the inoculation with members of Gigasporace. Indeed, Gigasporaceae performed better (i.e., TPC and antioxidant activity in fruits) than Glomeraceae but only in var.





Rio Grande. This is the first evidence that a taxonomically-based difference has been shown looking at functions (fruit quality) other than plant growth and P uptake. Previously, Hart and Reader (2002), studying the plant response of *Plantago lanceolata*, *Plantago major*, *Poa annua*, and *Poa pratensis* inoculated with a large number of AM fungal isolates, showed that AM fungal families differed significantly in their plant benefits (host biomass). However, they found

◂**Fig. 7** Dendrogram of similarity profile (SIMPROF) cluster analysis (horizontal dendrogram) grouping the different samples of tomato (*Solanum lycopersicum* L.) var. Pisanello (**a**) and var. Rio Grande (**b**) (roots collected at 2nd harvest). Plants were inoculated with AMF at nursery and mock inoculated (control, – M). Inocula were *Gigaspora gigantea* (*G.giga*) and *Scutellospora pellucida* (*S.pellu*) belonging to Gigasporaceae, and *Funneliformis mosseae* (*F.mos*) and *Sclerocystis sinuosa* (*S.sin*) belonging to Glomeraceae. Three field replicate plots per treatment. Samples are grouped into clusters based on their similarity/homogeneity of AM fungal intraradical communities (relative abundances of phylotypes). Vertical dendrogram groups the different phylotypes based on their similarity of occurrence. Red clusters are supported by the SIMPROF analysis

that Gigasporaceae, having the largest external mycelium, conferred less biomass promotion that Glomeraceae, having the largest internal mycelium. By contrast, similarly to our results in var. Rio Grande, they highlighted that Gigasporaceae acquired and transferred to the plant more P than Glomeraceae, but only in *P. major*. This supports the increased evidence of context dependency of the relation between AMF and the plant (Eom et al. 2000; van der Heijden et al. 1998). However, in addition to host specificity and intra- and extraradical mycelium traits of Gigasporaceae and Glomeraceae (Berger and Gutjahr 2021; de la Providencia et al. 2005; de Souza et al. 2005), other factors should be characterized to better explain the effect of AM fungal life-history strategy on plant response. For example, the AM fungal structures occurring within roots, such arbuscules and vesicles, and occurring in soil, such as absorptive hyphae and anastomoses, might be useful explanatory factors.

Moreover, in our study, within both AM fungal families, one isolate (i.e., *G. gigantea* and *S. sinuosa*) performed better than the other one for TPC and lycopene. This suggests that species belonging to the same family may show high functional variability. This supports previous finding on intra-family variation of plant growth and nutrient uptake (i.e., N and P) along with mycelium size and structure (Hart and Reader 2002; Munkvold et al. 2004). In addition to the intra-family functional variability, a little functional consistency was also previously found at species level (Avio et al. 2006; Börstler et al. 2008, 2010; Hart and Reader 2002; Koch et al. 2006; Mensah et al. 2015). Thus, the AM fungal variability at different taxonomic levels merits further investigation in order to better understand the complex relationship between AMF and their host plants in terms of synthesis of health-related secondary metabolites.

Health-related compounds in tomato sauce

The strong reduction observed, in this study, in TPC, antioxidant activity, and lycopene in the tomato sauce compared with fresh fruits highlights that the transformation process, based on thermal processing, draining and passing through a sieve negatively impact health-related compounds. Accordingly, Graziani et al. (2003) and Pavlović et al. (2017) reported that the antioxidant activity during thermal processing decreased compared to the value in fresh fruits of several tomato genotypes. Some studies demonstrated that lycopene is thermally stable in tomato-based food systems during mild thermal treatments (Khachik et al. 1992; Nguyen et al. 2001; Nguyen and Schwartz 1998; Thompson et al. 2000), while more intense thermal treatments can cause lycopene degradation reactions (Graziani et al 2003; Mayeaux et al. 2006; Sharma and Le Maguer 1996; Shi et al. 2003; Takeoka et al. 2001). Thus, these results support the need to develop new methods of transformation by adjusting temperature and processing time for preserving the nutraceutical quality of tomato sauce.

Overall, the increase of TPC, antioxidant activity and lycopene under inoculation with Gigasporaceae should be taken into consideration for the promotion and preservation of nutraceuticals in tomato sauce. This is the first evidence of an effect of Gigasporaceae on the concentration of secondary metabolites (nutraceuticals) in transformed products. In addition, the variability at intra-family level found in tomato fruits by our and other works (Hart and Reader 2002; Horsch et al. 2023) is here confirmed in the transformed product (sauce).

Diversity of AMF in roots of tomato plants

In this work, in accordance with Krüger et al. (2012), the phylogenetic information contained in the 3′ end of the SSU, the full ITS region and the 5′ end of the LSU (ca. 1500-bp-long fragment) was sufficient for discriminating at species level the diversity of AMF within the roots. Although this fragment does not cover the variable V4 and V5 regions of the SSU, commonly used in metabarcoding studies, and the assessment was based on a Sanger sequencing approach, the high number of screened clones per library (on average 56) together with the availability of a good number of public AM fungal sequences was sufficient to achieve robust data. The next-generation sequencing method based on Illumina platform would have allowed to sequence a higher number of reads per sample and to detect rare or low-abundance AM fungal taxa, providing a comprehensive view of the AM fungal community composition but the obtained short-reads would have allowed to assign sequences at phylogenetic resolution depending on the amplified gene region (genera or species) (Delavaux et al. 2022; Guzman et al. 2021; Higo et al. 2020; Mhlanga et al. 2022; Roy et al. 2017; Suzuki et al. 2020). By contrast, the third-generation long-read sequencing technology, such as the single molecule real-time sequencing (Pacific Biosciences, PacBio), would have enabled to obtain a lower sequencing depth compared to Illumina with an average length of 2.5 kb including the V4

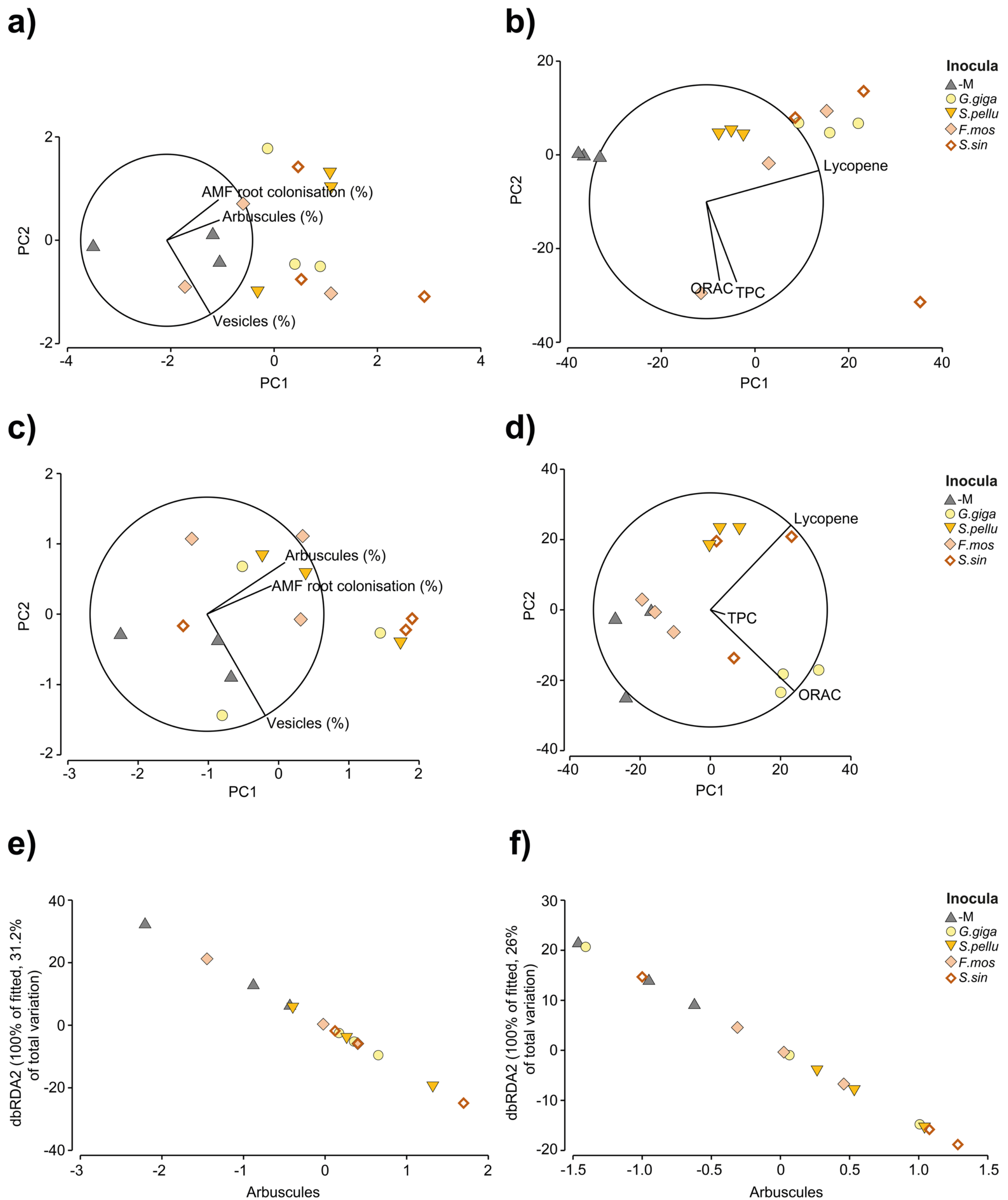


Fig. 8 Principal component analysis (PCA) plot on AM fungal colonization traits (arbuscules, vesicles, AM fungal root colonization) in the roots of tomato (*Solanum lycopersicum* L.) var. Pisanello (**a**) and var. Rio Grande (**c**) at 2nd harvest. Principal component analysis (PCA) plot based on quality parameters (total phenols, TPC; antioxidant activity, ORAC; lycopene) in fruits of var. Pisanello (**b**) and var. Rio Grande (**d**) at 2nd harvest. Distance-based redundancy analysis plot used to visualize the first and second axes of the distance-based linear method (DistLM) analysis applied to measure the significance and the variance explained by the best descriptor among the AM fungal colonization traits in var. Pisanello (**e**) and var. Rio Grande (**f**)

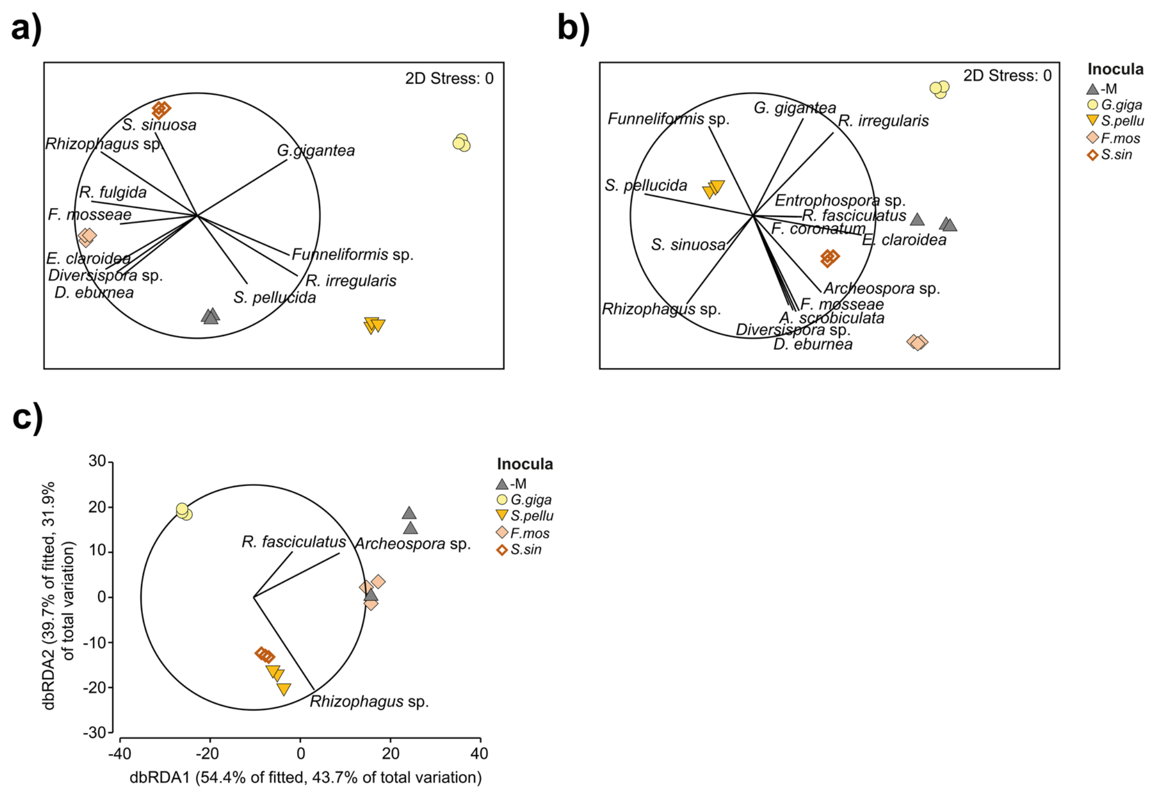


Fig. 9 Non-metric multidimensional scaling analysis (nMDS) plot based on AM fungal relative abundances of phylotypes retrieved within the roots of inoculated and not-inoculated tomato (*Solanum lycopersicum* L.) var. Pisanello (**a**) and var. Rio Grande (**b**). Distance-based redundancy analysis plot used to visualize the first and second axes of the distance-based linear method (DistLM) analysis applied to measure the significance and the variance explained by the best descriptor among the detected AM fungal phylotypes in var. Rio Grande (**c**)

and V5 regions of the SSU and providing a more efficient phylogenetic assignment (Kolaříková et al. 2021).

The molecular discrimination of the inoculated AM fungal species in the roots of both tomato varieties was certainly facilitated in our study by the fact that no AM fungal sequences affiliated to *G. gigantea*, *S. pellucida, F. mosseae*, and *S. sinuosa* were found in mock-inoculated controls. Indeed, in controls, the unique taxa phylogenetically close to the inoculated isolates was *Funneliformis* sp. close to inoculated *F. mosseae*. This could be determined by the fact that Gigasporaceae are adapted to live in stable and undisturbed ecosystems and are strongly reduced in tilled agricultural soils (de Souza et al. 2005; Farmer et al. 2007; Jansa et al. 2003). This depends on their peculiar life-history strategy that is characterized by the investment in somatic growth rather than in reproduction, development of large spore size and few offspring, and by a slow root infectivity rate. By contrast, members of Glomeraceae are commonly largely occurring in agricultural tilled soils due to their characteristic reproductive and colonising behavior and represented in our study up to 60% of the AM fungal community of control roots (var. Pisanello: *R. irregularis* 20%; *Funneliformis* sp. 31%; var. Rio Grande: *Rhizophagus* sp. 13%, *R. irregularis* 12%, *R. fasciculatus* 7%, *Funneliformis* sp. 18%, and *F. coronatus* 10%). Similarly, we found members of Glomeraceae largely occurring within the roots of inoculated treatments (on average 71% in var. Pisanello and 57% in var. Rio Grande). *Funneliformis* sp. was consistently present in inoculated treatments, according to its well-known generalist behavior (Moora et al. 2011; Öpik et al. 2009). The increased incidence of Glomeraceae in tilled soils has been explained by a stimulatory effect of tillage that increases the number of hyphae and colonized root fragments, acting in this AM fungal family as infective propagules, and by a lower competition for root colonization (Boddington and Dodd 2000; Jansa et al. 2003). Moreover, among Glomeraceae, *S. sinuosa* has been generally found at low abundance in agricultural soils (Balestrini et al. 2010; Liu et al. 2016; Turrini et al. 2017). This could explain the presence and high abundance of this AM fungal species in the plants of both tomato varieties inoculated by *S. sinuosa*.

The hypothesis that life-history strategies of Gigasporaceae and Glomeraceae would have differentially affected the AM fungal diversity and communities within the roots of tomato was confirmed by our results. However, members of Glomeraceae that we would expect to greatly increase the

AM fungal biomass inside the roots as compared to Gigasporaceae (Hart and Reader 2002; de Souza et al. 2005) and thus strongly affect root assemblages, showed an AM fungal diversity and community like control. By contrast, members of Gigasporaceae that are expected to produce low AM fungal biomass inside the roots (Hart and Reader 2002; de Souza et al. 2005), in our study were found to reduce the AM fungal diversity and strongly modified the community in roots compared to control. These findings are supported by the data on AM fungal root colonization reported by Arcidiacono et al. (2023). Indeed, AM fungal inoculation increased root colonization irrespective of the AM fungal families in both tomato varieties. Thus, following inoculation of foreign AM fungal taxa, the competition between native AMF and inoculated taxa can be considered an important driver of the AM fungal diversity and communities in roots. A stronger competitiveness of Gigasporaceae members is evidenced by the higher relative abundance of these taxa within the roots of the varieties Pisanello and Rio Grande in comparison to Glomeraceae (up to 50% with *S. pellucida* in var. Rio Grande).

Functional relationship between AM fungal traits and tomato quality parameters

Following the results of Arcidiacono et al. (2023), AM fungal root colonization, arbuscules, and vesicles in var. Pisanello were significantly higher in inoculated plants than controls (88%, 360%, and 66%, respectively), and an intra-family variability in AMF root colonization of Glomeraceae was found (i.e., *S. sinuosa* had higher AM fungal root colonization than *F. mosseae*: 49%). Moreover, in var. Rio Grande, the percentage of root length containing vesicles was significantly increased by inoculation (51%), and vesicles were higher in Gigasporaceae than Glomeraceae (13%) and in *F. mosseae* than *S. sinuosa* (27%). Similarly, AM fungal root colonization was higher in plants inoculated with *F. mosseae* than *S. sinuosa* (22%). By relating the AM fungal colonization traits (i.e., arbuscules, vesicles and AM fungal root colonization) within the roots of the two tomato varieties with the corresponding fruit quality parameters (i.e., TPC, antioxidant activity, and lycopene), the change in arbuscules was sufficient to describe the pattern of fruit quality parameters. Thus, we can infer that the main determinant of the improved host benefit in Gigasporaceae-inoculated plants of var. Rio Grande is the higher occurrence of arbuscules. Indeed, inoculation by Gigasporaceae of var. Rio Grande was reported to improve, at transplanting, the occurrence of arbuscules and to increase, at harvest, nutrient concentrations in fruits compared to Glomeraceae (Arcidiacono et al. 2023). Since arbuscules are the well-known site of carbon-mineral exchanges between host and fungus (Luginbuehl et al. 2017; Parniske 2008), it is not surprising that they play this major role also in secondary metabolite synthesis by the host.

Moreover, by relating the AM fungal community in roots of the two tomato varieties with the corresponding fruit quality parameters, in var. Rio Grande, the changes of some native taxa (i.e., *R. fasciculatus*, *Rhizophagus* sp. and *Archeospora* sp.) were the best predictors of the pattern of quality parameters, whereas in var. Pisanello quality parameters could not be described by any AM fungal phylotypes. Thus, the main driver of the improved host benefit in var. Rio Grande is not the identity of the inoculated AM fungus, but the competition between the introduced isolate and the native AM fungal community that determines changes in the abundance of some native taxa in roots. This is in agreement with recent findings obtained by relating the AM fungal assemblages within the roots of alfalfa with corresponding plant traits (Pellegrino et al. 2022). In this work, the change in abundance of one local isolate of *R. irregularis* induced by all inoculation treatments was sufficient to describe the pattern of plant traits. Overall, our results suggest an environmental-driven selection for highly efficient AMF and support the use of Gigasporaceae as inoculants for enhancing the nutritional value of tomato.

Conclusions

Our work underlines the key role played by AM fungal inoculation of tomato at nursery for improving the quality parameters of fruits. The differences detected between AM fungal families in the benefit to tomato in var. Rio Grande highlight that host response, driven by life-history strategy, depends also on plant host genotype. Moreover, given the high reduced concentration of nutraceuticals in thermal-processed products, such as the tomato sauce, nursery application of selected highly efficient inoculants (i.e., Gigasporaceae) may results in a great support for the quality of the final transformed products. Nevertheless, the technology of transformation could be improved in order to optimize the beneficial outcome of the AM fungal symbiosis. Gigasporaceae were also found to be more competitive with native AMF, modifying the diversity and community in the roots of tomato, as compared to Glomeraceae. This competitive behavior, shown to be beneficial for the quality of tomato, might be risky for biodiversity losses due to bio-invasion of exotic inoculants. However, the relationship between arbuscules and fruit quality parameters in the tested tomato varieties as well as the relationship between some native AMF and the fruit quality parameters of var. Rio Grande should be verified in controlled conditions excluding confounding factors. Finally, the intra-family functional variability detected for nutraceutical compounds suggests that AM fungal family alone might not be a sufficient predictor of the host response

and that further investigations are needed to better characterize the role played by intra-family variability in the outcome of symbiosis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-023-01778-6.

Acknowledgements The authors would like to thank Alessandro Agostini Venerosi della Seta for allowing to run the field experiments at the "Fattoria Le Prata" farm (Pisa, Italy).

Author contribution EP and LE acquired funding, conceived, and designed the study. MA carried out the experimental procedures. EP and MA performed the molecular study, collected the data, performed the statistical analyses, and wrote the first draft of the manuscript. AF set-up the extractions and following analysis of antioxidant activities. LE and AF contributed to the discussion of the data. All authors commented on the manuscript and approved the final version.

Funding Open access funding provided by Scuola Superiore Sant'Anna within the CRUI-CARE Agreement. This work was funded by the the European Agricultural Fund for Rural Development 2007–2013 for Tuscany (Italy), measure 16.1 (FERTIBIO project; CUP Artea 743548) project leader Dr. Elisa Pellegrino and measure 16.2 (FERTIBIO project; CUP Artea 828090) project leader Prof. Laura Ercoli. Myriam Arcidiacono was supported by a PhD scholarship from Scuola Superiore Sant'Anna (SSSA, Pisa, Italy).

Data Availability Data used in this study can be made available upon reasonable request following data-sharing regulations.

Declarations

Competing interests The authors declare no competing interests.

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