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Short communication

Accumulation of flavonoids and phenolic compounds in olive tree roots in response to mycorrhizal colonization: A possible mechanism for regulation of defense molecules

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ABSTRACT

The arbuscular mycorrhizal (AM) fungus promotes plant growth and can alter the production of primary and secondary metabolites. The aim of this work was to determine the influence of AM fungi colonization on the content of phenolic compounds, flavonoids and soluble carbohydrates in olive (*Olea europaea* L.) tree roots. The results revealed that mycorrhizal plants had a higher content of flavonoids and total phenols. Analysis of sugar contents showed enhanced levels of sucrose and fructose in mycorrhizal roots, while glucose amounts stayed constant. The DPPH radical-scavenging activity of the mycorrhizal root methanolic extracts was higher than that of the non- mycorrhizal root methanolic extracts. These results indicated that olive tree roots contain significant amounts of phenolic compounds, important factors for antioxidant capacity, which can be substantially modified by colonization of olive trees with AM fungi.

1. Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous biotic components in terrestrial ecosystems, readily establishing symbiosis with over 80% of land plant species (Smith and Read, 2008). AM fungi are well known to improve plant nutritional status by enhancing the uptake of essential nutrients such as phosphorous and nitrogen and by improving the water supply through an increase in root surface area (Smith and Read, 2008). In return, AM fungi receive from the plant carbohydrates that are needed for energy metabolism, maintenance and growth (Bago et al., 2000). Laboratory and field studies have shown that plants receive a variety of benefits from AM fungi. Benefits include increased survival, nutrient uptake, growth, and reproduction (Smith and Read, 2008; Zhang et al., 2011; Ferrazzano and Williamson, 2013). Furthermore, other functions of AM fungi have been continually shown, such as increasing the resistance to pathogens (Khan et al., 2010; Wehner et al., 2010). Several basal defense mechanisms contribute to the increased resistance by AM fungi (Fontana et al., 2009; Wehner et al., 2010), among which the promoted phenolic synthesis is the focus of interest (Yao et al., 2007; Khan et al., 2010; Mandal et al., 2010). Considerable increases in phenolic compounds in host plants as a result of AM fungus inoculation have been reported (Oliveira et al., 2013). Generally, inoculation of plants with AM fungi results in an overall increase in the production of some new phenolic compounds during the progression of the infection (Devi and Reddy, 2002).

In plants, sucrose constitutes the main form of carbohydrate for long-distance transport. This disaccharide is synthesized in the photosynthetic source leaves and is then loaded into the phloem sap, where sucrose follows its route through the transport phloem. Finally, sucrose is unloaded to supply sink tissues such as roots (Doidy et al., 2012). Investigations on the carbohydrate metabolism showed that sucrose synthase and invertase activities or expression of the corresponding genes are increased in mycorrhizal roots (Schaarschmidt et al., 2006; Boldt et al., 2011). Plant invertases including acid invertase and neutral invertase cleave sucrose to both glucose and fructose, and sucrose synthase cleaves sucrose to fructose and UDPG (uridine diphosphate glucose) (Schubert et al., 2004). Nuclear magnetic resonance spectrometry demonstrated that AM fungi take up hexoses, primarily glucose, not the disaccharide sucrose and the hexose fructose (Bago et al., 2000). On the other hand, glucose is involved in the synthesis of a major-

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ity of the phenolic compounds and flavonoids in plants (Mandal et al., 2010). These results indicate that carbohydrate metabolism is enhanced, leading to higher hexoses levels in mycorrhizal roots, where they are probably used for flavonoid and phenolic compound synthesis and/or consumed by the mycorrhizal fungus needed for energy metabolism, maintenance and growth. This can have positive effects on plant performance: (i) Flavonoids and phenolic compounds are immensely important in plant-microbe interactions/symbiosis and act as agents in plant defense (Khan et al., 2010; Mandal et al., 2010); (ii) AM fungi are known to stimulate host plant growth, mainly by enhancing soil nutrient uptake (Kothari et al., 1990).

Olive (*Olea europaea* L.) trees are known to form arbuscular mycorrhiza (Porras-Soriano et al., 2009; Mechri et al., 2014). It has been shown that AM fungi improve the growth, water relations, nutrient uptake, photosynthesis and tolerance to salinity in olive trees (Porras-Soriano et al., 2009). Early inoculation of olive seedlings enhances early plant development and crop productivity of olive trees (Estaún et al., 2003). The inoculation of olive plantlet cultivars 'Koroneiki' and 'Valanolia' significantly increased the plantlet height, stem diameter, number of lateral shoots and leaves, internode length, and leaf total phenols, chlorophylls and carotenoids (Seifi et al., 2014). In cultivated olives, AM inoculation in the cultivar 'Mission' increased root and leaf phenolic contents (Ganz et al., 2002). However, Espinosa et al. (2014) found no effect of colonization of olive seedlings by AM fungi on root biosynthesis of phenolic compounds.

The objective of our study was to determine the influence of mycorrhizal inoculation with the AM fungi *Rhizophagus irregularis* on the contents of total phenols, flavonoids and soluble carbohydrates in olive tree roots. The antioxidant activity of olive root methanolic extracts was also evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay.

2. Materials and methods

2.1. Experiment description

The experimental design used in this work has been described previously (Mechri et al., 2014). Briefly, spores of *Glomus intraradices* DAOM 197198, now *R. irregularis* DAOM 197198 (Krüger et al., 2012), used in this study were inoculated in a sample of olive plantlets (15 cm long and three pairs of leaves). After 2 weeks of acclimatization in a greenhouse, the olive plantlets were potted into individual 10 l pots of 20 cm in diameter (one plant per pot) filled with a sandy P-poor soil (pH 7.68; sand: 91.6%; silt: 1.5%, clay: 6.9%, Ctot: 4.3 g kg^{-1}, Olsen-P: 8.38 mg kg^{-1}) collected directly from an olive tree field. At the time of re-potting, 1000 spores of *R. irregularis* were deposited directly below the roots of each plantlet. The experiment was conducted in a fully randomized block design with two treatments and three replications. Treatments consisted of non-mycorrhizal plant (NM) and mycorrhizal plant with *R. irregularis* (M). The experiment was carried out under controlled greenhouse conditions. The average air temperature in the greenhouse was 25–30 °C. Plants were grown under natural light. Plants were watered every second day to maintain a soil water level corresponding to 65% of the field capacity. Six months after planting, plants were harvested and root samples were collected from each treatment.

2.2. Determination of glucose, fructose and sucrose in roots of M and NM olive trees

Glucose, fructose and sucrose in roots of M and NM plants were determined according to the method described previously (Mechri et al., 2011). Briefly, glucose, fructose and sucrose from composite root samples were extracted twice in 80% ethanol at 70 °C. Extracts were dried and converted into trimethylsilyl ethers with a silylation mixture made up of pyridine, hexamethyldisilazane and trimethylchlorosilane. Glucose, fructose and sucrose were analyzed using a Hewlett–Packard 5890 series II gas chromatograph equipped with a flame ionization detection (FID) system and a HP-5MS capillary column (30 m × 0.25 mm). Injector and detector temperatures were 280 °C and 300 °C respectively. The following temperature program was set: 80 °C for 1 min, from 80 to 170 °C at 10 °C/min, from 170 to 200 °C at 15 °C/min, from 200 to 315 °C at 25 °C/min and finally 315 °C for 8 min. Identification of glucose, fructose and sucrose was achieved by the use of the relative retention times, i.e., in comparison to that of the trimethylsilyl derivates of standard carbohydrates.

2.3. Extraction of flavonoïds and phenolic compounds

1 g from M and NM fresh olive roots was extracted in 10 ml of methanol on a shaker at 200 rpm for 24 h. After centrifugation (5000 × *g* for 10 min), all extracts obtained were then transferred to vials and kept in the dark at −20 °C. The extracts were filtered thought a 0.45 μm syringe filter prior to analysis (Taamalli et al., 2012).

2.4. Determination of total phenols and flavonoïds in roots of M and NM olive trees

Total phenolic contents of M and NM roots were determined according to the method of Montedoro et al. (1992) with slight modifications. 0.4 ml of root extracts and 10 ml of diluted Folin–Ciocalteu reagent were mixed. After 1 min incubation, 8 ml of sodium carbonate (75 g/L) was added and the mixture was incubated for 1 h. The absorbance was measured at 765 nm. Total flavonoid contents in the roots of M and NM olive tree were determined according to the method of Zhishen et al. (1999). One ml of methanolic root extract was mixed with 4 ml of distilled water. At zero time, 0.3 ml of (5%, w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10%, w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH were added. Finally, the volume was made up to 10 ml, immediately by the addition of 2.4 ml of distilled water. The mixture was shaken vigorously and the absorbance was read at 510 nm.

2.5. DPPH radical-scavenging activity

Scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured according to the procedure described by Blois (1958) with slight modifications. 20 μl from the M and NM methanolic root extracts were dissolved in absolute methanol to a final volume of 1 ml and then added to 1 ml DPPH (0.1 mM, in absolute methanol). The absorbance of the mixture was measured at 517 nm after 30 min of incubation at 37 °C in the dark. DPPH radical scavenging activity was expressed as the inhibition percentage which was calculated as absorbance of control minus absorbance of sample/absorbance of control ×100.

2.6. Statistical analysis

The data obtained with measurement of the concentrations of glucose, fructose sucrose, phenolic compounds and flavonoids of olive tree roots were statistically analyzed using SPSS statistical software version 10.0. The significance of differences between mean values was determined by one-way analysis of variance. Duncan's multiple range test was used to compare the means. The significance probability levels of the results are given at the $p < 0.05$ level.