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In vitro micropropagation and mycorrhizal treatment influences the polyphenols content profile of globe artichoke under field conditions

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ABSTRACT

The commercial importance of plant tissue culture has grown in recent years, reflecting its application to vegetative propagation, disease elimination, plant improvement and the production of polyphenols. The level of polyphenols present in plant tissue is influenced by crop genotype, the growing environment, the crop management regime and the post-harvest processing practice. Globe artichoke is a significant component of the Mediterranean Basin agricultural economy, and is rich in polyphenols (phenolic acids and flavones). Most commercially grown plants are derived *via* vegetative propagation, with its attendant risk of pathogen build-up. Here, a comparison was drawn between the polyphenol profiles of conventionally propagated and micropropagated/mycorrhized globe artichoke plants. Micropropagation/mycorrhization appeared to deliver a higher content of caffeoylquinic acids. The accumulation of these compounds, along with luteolin and its derivatives, was not season-dependent. Luteolin aglycone was accumulated preferentially in the conventionally propagated plants. Overall, it appeared that micropropagation/mycorrhization enhanced the accumulation of polyphenols.

1. Introduction

The *in vitro* propagation of plant tissue has contributed significantly to crop improvement by facilitating the production of pathogen-free planting materials, as well as by providing a means of enhancing the production of pharmaceutically active secondary metabolites (Hussain, Qarshi, Nazir, & Ullah, 2012). The added cost of using micropropagated planting material is often more than compensated by an improved field performance (Rey, Papacchioli, Tavazza, & Pagnotta, 2013).

The *Cynara cardunculus* complex includes three recognized species, all native to the Mediterranean Basin; its members are the globe artichoke (var. *scolymus*), the cultivated cardoon (var. *altilis*) and the wild cardoon (var. *sylvestris*). The globe artichoke is cropped for both culinary and medicinal purposes (Cefola et al., 2012; Lattanzio, Kroon, Linsalata, & Cardinali, 2009), due its high content of caffeoylquinic acids and flavones (Dabbou et al., 2015). Roberfroid (2000) has even suggested that it should be considered as a functional food. The global production of the harvested portion of the globe artichoke plant (the head or *capitulum*) is rising (Faostat, 2013). The major share of production is situated in Italy, where many landraces are cultivated. Therefore, an enriched globe artichoke might be spread its consumption also over Mediterranean Basin. These landraces are almost exclusively propagated vegetatively, thereby retaining a high level of population heterozygosity, which has facilitated clonal selection aimed at improving a number of both morphological and nutritional traits (Pandino, Lombardo, Mauro et al., 2012; Portis, Barchi, Acquadro, Macua, & Lanteri, 2005). A major disadvantage of this mode of propagation is that it fails to control the accumulation of pathogens, which could be affect the commercial production leading to a major use of pesticides (Ancora, 1986). A potential solution to this problem, which at the same time retains the characteristic heterozygosity of the material, is to replace conventional vegetative propagation with micropropagation (El Boullani, Elmoslih, El Finti, El Mousadik, & Serghini, 2012; Morone Fortunato, Vanadia, & Lattanzio, 1981). Some concern has been expressed that the phenotype of micropropagated plants may not fully match that of the donor, as a result of somatic mutagenesis occurring during the *in vitro* multiplication stage (Devarumath et al., 2002).

The polyphenol profile of plant tissue is influenced by the plant's genotype, its growing environment, the management regime applied and the post-harvest processing practices carried out (Beckman, 2000). Recently it was reported as mycorrhizal inoculation may represent an efficient and sustainable strategy to enhance plant phenolic compounds in globe artichoke plants (Palermo, Colla, Barbieri, & Fogliano, 2013).

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Nevertheless,the effect (if any) of micropropagation on the polyphenol profile of globe artichoke plants raised *via* micropropagation (along with mycorrhization) has not as yet been determined, although some reports have appeared in the literature regarding its influence over agronomic performance and the content of total polyphenols, sugar and organic acids (Cappelletti et al., 2016; Cavallaro, Barbera, Castiglione, Scandurra, & Longo, 2013; Pandino, Meneghini, Tavazza, Lombardo, & Mauromicale, 2017). Therefore, the aim of this paper was to examine profile changes to both caffeoylquinic acid and flavones in globe artichoke in response to micropropagation/mycorrhizal treatment. The plants used here were assessed over two growing seasons to establish the environmental stability of changes induced by micropropagation/mycozzhization.

2. Material and methods

2.1. Chemicals

Reagents and solvents were purchased from VWR (Leighton Buzzard, UK) and were of analytical or HPLC grade. Apigenin-7-*O*-glucoside, apigenin, luteolin-7-*O*-glucoside, luteolin, 5-*O*-caffeoylquinic acid (chlorogenic acid), and hesperetin were obtained from Extrasynthese (Lyon, France), cynarin (1,3-di-*O*-caffeoylquinic acid) was from Roth (Karlsruhe, Germany). Milli-Q system (Millipore Corp., Bedford, MA) ultrapure water was used throughout this research.

2.2. In vitro culture of globe artichoke explants

Three clones of globe artichoke 'Violetto di Sicilia' mother plants (C1, C2 and C3) were selected to establish *in vitro* micropropagation. From each mother plant, 15 offshoots were picked up and shoot tips, 5–6 mm in length, were excised to become primary explants. They were surface sterilized by immersion in 70% ethanol (v/v) for 1 min followed by 0.05‰ mercuric chloride solution for 15 min and washed three times with sterile distilled water. Then the explants were transferred into 70 mL glass tubes containing 20 mL of basal medium (BM) supplemented with 6-(γ,γ-dimethylallylamino)purine (2iP) (1 mgL^{-1}), indoleacetic acid (IAA) (1 mgL^{-1}) and gibberellic acid (GA3) (0.025 mgL^{-1}) to establish the *in vitro* conditions (Morone-Fortunato, Ruta, Castrignano, & Saccardo, 2005). The basal medium (BA) was composed by Murashige and Skoog (1962) macronutrients, Nitsch and Nitsch (1969) micronutrients, Ferric EDTA (30 mg L^{-1}), thiamine HCl (0.4 mg L^{-1}), myoinositol (100 mg L^{-1}), agar (7 g L^{-1}) and sucrose (20 g L^{-1}). After 3 weeks, the shoots were transferred into 200 mL glass vessels containing 40 mL of proliferation medium composed of BM and 0.05 mg L^{-1} 6-benzylaminopurine (BAP) (Morone-Fortunato et al., 2005) and subcultured three times each 25 days. The grown shoots were rooted for 30 days in the same kind of glass vessels containing 40 mL of BM added with a higher sucrose concentration (30 g L^{-1}) and supplemented with IAA (10 mg L^{-1}) (Morone-Fortunato et al., 2005). During all the *in vitro* culture, globe artichoke explants were maintained in a growth chamber at 22 ± 2 °C with a photoperiod of 16 h light and under a light intensity of 60 $\mu Em^{-2} s^{-1}$. At the time of transplant, the uniform rooted microplants, 40 for each clone, were inoculated with 10 g of crude arbuscular mycorrhiza fungus inoculum of *Glomus viscosum* H.T. Nicolson (syn. *Septoglomus viscosum*). The inoculum consisted of infested soil containing spores (about 50–60 spores in 10 g of inoculum), external mycelium and infected root fragments obtained from strawberry pot-cultures. It was added below the root system to each pot (v = 0.2 dm^3) filled with sterilized commercial peat mixture enriched with nutrients (organic carbon 46%, organic nitrogen 1–2%, organic matter 80%) and mixed with perlite at a 2:1 (v/v) ratio.

Acclimatization took place at the University of Bari "Aldo Moro", Apulia, Italy (41° 7′ 31″ N, 16° 52′ 0″ E) in greenhouse condition at 20–25 °C with mist, reducing the humidity level from 85 to 90% to 55–60% over 20 days.

2.3. Experimental plots and sampling

Field experiment was conducted at the experimental station of Catania University, on the coastal plain of Cassibile (SR) (36°58′N; 15°11′E; 53 m a.s.l.), a typical area for globe artichoke cultivation in Italy. The local climate consists of mild and wet winters and hot, dry summers. The plant material [micropropagated/mycorrhized plants and semi-dormant offshoots ("ovoli") for vegetative propagation] was planted in August 2011 and arranged in a randomized block experimental design with four replicates. Each field plot consisted of ten plants, spaced 0.80 m apart with row spacing of 1.25 m. Crop management (fertilization, irrigation, weed and pest control) was performed according to the standard local commercial practice. At least 5 capitula, including the floral stem, per replicate, at the usual marketing stage, regardless of their size, were harvested disease-free in February–March and washed with tap water. At this stage, the length of the central global flower buds was ≤ 2 mm. They were combined, cut and then blended using a domestic food processor at 0 °C (Kenwood multipro, Milan, Italy). Finally, an amount of sample was freeze–dried and stored at − 20 °C until analysis.

2.4. Extraction procedure and HPLC analysis

The extraction procedure and HPLC analysis for polyphenols profile was carried out as described by Pandino, Courts, Lombardo, Mauromicale, and Williamson (2010). Each extract was analysed using a series 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA) equipped with ChemStation software (B.03.01) and a diode array detection system. Separations were achieved on a Zorbax Eclipse XDB-C18 (4.6 × 150 mm; 5.0 μm particle size), operated at 30 °C, with a 0.2 μm stainless steel inline filter.

The HPLC conditions were: the mobile phase was 1% formic acid in water (solvent A) and in acetonitrile (solvent B) at a flow rate of 0.5 mL/min. The gradient started with 5% B to reach 10% B at 10 min, 40% B at 30 min, 90% B at 50 min, 90% B at 58 min. Chromatograms were recorded at 280, 310, and 350 nm from the diode array and data were collected between 200 and 600 nm. Each compound was identified based on its retention time, UV spectrum and already published identification of compounds from globe artichoke (Schütz, Kammerer, Carle, & Schieber, 2004; Wang et al., 2003). Quantification was performed by a calibration curve using the available standards. Mono- and dicaffeoylquinic acids (CQA) were calculated using 5-*O*-CQA and 1,3-*O*-diCQA as references, respectively. Apigenin (API) and luteolin (LUT) conjugates were quantified as API-7-*O*-glucoside and LUT-7-*O*-glucoside, respectively. All data presented are mean values ± standard deviation of three independent experiments (n = 3) and expressed as g kg^{-1} of dry matter.

2.5. Statistical analysis

All data were subjected to analysis of variance (ANOVA) and means were separated by LSD (least significant difference) tests, when the F-test was significant.

3. Results and discussion

Here, differences in the polyphenol content were analysed by comparing conventionally propagated and micropropagated/mycorrhized planting material of three distinct globe artichoke clones. The latter plants were inoculated with the arbuscular mycorrhizum species *G. viscosum* (Campanelli, Ruta, Tagarelli, Morone Fortunato, & De Masro, 2013).

Sbrana, Avio, and Giovannetti (2014) have recently reviewed the evidence for a positive effect of mycorrhizal treatment on the production of certain health-promoting phytochemicals. The implication is that the effect on polyphenol accumulation ascribed to