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Field performance of micropropagated and mycorrhizal early globe artichoke plants

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ABSTRACT

Early traditional varieties of globe artichoke are a profitable crop in southern Mediterranean regions. One of the main issues is the availability of propagative plant material that can ensure homogeneous and early production. This paper evaluates the micropropagation technique in association with mycorrhizal inoculation in terms of obtaining high-quality propagative material for *Cynara cardunculus* L. var. *scolymus* Fiori cultivar "Violet de Provence". To confirm the morphological and productive performance of these plants, their agronomical assessment was studied under field conditions. In addition, the effects of mycorrhizal symbiosis were examined on the nutritional quality of artichoke heads. The results showed that micropropagated plantlets were healthy and homogeneous. The inoculation with *Septoglomus viscosum* improved the growth and development of the plantlets, alleviating the stress of post-transplanting. In the field, the micropropagated and mycorrhizal plants and the heads exhibited the standard morphological characteristics of the cultivar "Violet de Provence". The productivity performance of the heads highlighted the positive effects of mycorrhizal symbiosis on the weight, earliness and total yield. Finally, arbuscular mycorrhizal (AM) fungus also had a very positive influence on the nutritional quality of the heads, in terms of essential macro-mineral, total phenolic and inulin content. Micropropagation and mycorrhizal techniques appeared to be an efficient and sustainable strategy for improving the yield and nutritional quality of globe artichoke heads under field conditions.

1. Introduction

The globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori) is a herbaceous perennial plant belonging to the *Asteraceae* family. It is widely cultivated in the Mediterranean for its immature inflorescences commonly called heads or capitula, which are rich in bioactive phenolic compounds, minerals and inulin (Fratianni et al., 2007; Lattanzio et al., 2009; Lombardo et al., 2010; Pandino et al., 2011a, 2011b; de Falco et al., 2015). Cultivated varieties are classified as early or reflowering and late varieties, according to their harvesting time. The early varieties begin to produce edible heads in autumn, the late varieties only produce in late winter or early spring depending on the photo and thermoperiods. The usual way to propagate this species is clonal propagation starting with offshoots, underground dormant axillary buds, common called "ovoli", or divisions from mature plants. This method has many disadvantages such as the physiological heterogeneity of the plantlets, the low rate of multiplication, and the spread of pathogens (Cardarelli et al., 2005; Lanteri and Portis, 2008; Ceccarelli et al., 2010). Micropropagation can thus be very suitable in order to obtain genetically stable, pathogen-free plantlets with a high commercial quality in less space and time than other methods (George et al., 2008; Akin-Idowu et al., 2009). However, although late varieties of globe artichoke are successfully multiplied by this technique, early varieties are more recalcitrant and an effective protocol has yet to be found. In fact, the phenotypical and behavioural variations resulting in the field can lead to a loss of earliness of the first harvest and consequently a lack of interest in using this propagation method for the reflowering varieties of artichoke.

Using arbuscular mycorrhizal fungi (AMF) during the acclimatization stage of micropropagation ensures greater survival, better development of the roots and a more rapid growth of micropropagated early artichoke plantlets (Morone Fortunato et al., 2005; Campanelli et al., 2014), as also shown by several studies on other species (Kapoor et al., 2008; Akin-Idowu et al., 2009; Chandra et al., 2010; Wu et al., 2011). AMF develop a symbiotic relationship with the host plant and help them to overcome transplant stress and reduce the acclimatization period. This then increases the nutrient uptake, water conducting capacity and photosynthetic rates, as well as protecting the plant from root diseases (Salamanca et al., 1992; Azcon-Aguilar and Barea, 1996; Kapoor et al., 2008). The plants also become more tolerant to biotic and

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abiotic stresses, and consequently enhance the yield (Sylvia and Williams, 1992; Auge, 2001; Campanelli et al., 2013). Inoculated plants in the field may therefore lead to an ecological production system that reduces the need for fertilizer and chemical applications, and increases quality and yield (Rouphael et al., 2015). Most experiments conducted so far have been in the controlled microcosm conditions of the greenhouse. Only a few studies have examined the behavior of the inoculated plant after transplantation in open fields where the natural system may modify the plant-mycorrhiza interaction and thus the plant responses (Rouphael et al., 2015).

This study was thus carried out in the field. The aim was to evaluate micropropagated and mycorrhizal plants of the *Cynara cardunculus* L. var. *scolymus* Fiori cultivar "Violet de Provence" in order to establish the efficiency of these techniques in terms of morphology and productivity. In addition the effects of mycorrhizal symbiosis were also examined on the essential macro-mineral (sodium, potassium, calcium and magnesium) profile, polyphenol and inulin contents to establish the variation in nutritional quality of artichoke heads.

2. Material and methods

2.1. Plant production

2.1.1. Micropropagation, multiplication of AM fungi and mycorrhizal inoculation

Globe artichoke plantlets (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori), cv Violet de Provence were obtained by micropropagation from offshoot explants following the protocol described by Morone Fortunato et al. (2005). At the same time, spores of AM pure cultures were multiplied for four months in a greenhouse located at the Department of Agricultural and Environmental Science - University of Bari "A. Moro", southern Italy (41° 7′ 31″ N, 16° 52′ 0″ E) according to Dalpé and Monreal's method (2004). Strawberry (*Fragaria* x *Ananassa*) plants were used as the trap crop due to the high mycotrophy, and sterile sand was used as the substrate. In order to evaluate the effects of symbiosis on the acclimatization to *ex vitro* conditions, at the time of the transplantation in the greenhouse 100 rooted globe artichoke microplants with crude AM fungus *Septoglomus viscosum* (Basionym: *Glomus viscosum* T.H. Nicolson (1995)) were inoculated. For the inoculation, approximately ten grams (about 100–120 spores) of AM fungal inoculum were placed immediately below the roots. The AM fungal inoculum consisted of sand soil that contained spores, external mycelium and infected strawberry root fragments. The remaining plants (100) were not inoculated with AM fungus and were used as controls. Acclimatization was performed using 8 cm plastic pots containing a sterile peat (46% organic carbon, 1–2% organic nitrogen, 80% organic matter) and a perlite mixture (2:1, v/v ratio) at 18–25 °C with mist, reducing the humidity level from 85 to 90% to 50–60% over 20 days. The plants were then grown at 20 °C at 40% relative humidity (RH).

The trial was arranged in a randomised complete design with 100 replicates for each treatment (inoculated and non-inoculated plants).

2.1.2. Biometric, physiological and symbiotic parameters

Three months after the inoculation, morphological parameters, leaf number per plant, leaf area (Licor LAI Area Meter 3100), fresh and dry weight of shoots and roots, root density, were measured on 20 plants chosen at random both from mycorrhizal inoculated plants and controls without mycorrhiza. Symbiotic parameters (percentage mycorrhizal colonization) (Trouvelot et al., 1986) and dependency were also quantified. Mycorrhizal dependency (MD) was calculated using the individual total dry weight (DW) of mycorrhizal plants (MP) and the mean dry weight of non-mycorrhizal plants (non-MP), according to Plenchette et al. (1983) as: MD = DW_{MP}- $DW_{non\text{-}MP}$ /DW_{MP} x 100. In addition, SPAD values (Minolta Chlorophyll Meter SPAD-502 Plus) were monitored between 8:00 and 10:00 on the first fully-expanded leaf with three readings repeated. The main essential macro-minerals (sodium, potassium, calcium and magnesium) related to health properties of globe artichoke were analysed in leaf tissue dry matter (DM) in order to study the influence of the symbiosis on their uptake. Three randomly selected leaves per replicate were dry-ashed at 550 °C for 4 h, mixed with hot 1 M HCl, filtered, and then brought to a final volume of 50 ml with distilled water according to the official AOAC method (1995). The concentrations of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were measured by ion chromatography (761 Compact IC Metrohm).

2.2. Agronomic evaluation in the field

2.2.1. Trial location, climate and soil

The agronomic evaluation of acclimatized plantlets, inoculated with *S. viscosum* or not inoculated, was carried out during two cropping seasons (2011–2013) at the experimental farm of Bari University and located in Policoro (MT, southern Italy; 40°10′20″ N, 16°39′04″ E). This site is 15 m above sea level and is characterized by a Mediterranean climate according to the De Martonne classification (Cantore et al., 1987) with an average annual rainfall of 560 mm distributed mainly during autumn and winter. The soil is more than 1.2 m deep and has a standard loam texture according to the physical characteristics: sand 398 g kg^{-1}, silt 374 g kg^{-1}, clay 228 g kg^{-1}. The chemical characteristics of the soil were: pH 7.7; total N (Kjeldahl method) 1.7 g kg^{-1}, available P (Olsen method) 27.6 mg kg^{-1}, exchangeable K_2O (ammonium acetate method) 227 mg kg^{-1}, organic matter 2.3% (Gazz, 1999), total carbonate 15.0 g kg^{-1}, active carbonate 5.0 g kg^{-1}, saturated paste extract electrical conductivity (ECe) 0.95 dS m^{-1}, exchangeable Na percentage (ESP) 1.9%; bulk density 1.25 kg dm^{-3}; soil moisture at field capacity (measured *in situ*) 0.32 m^3 m^{-3} and at wilting point (−1.5 MPa) 0.15 m^3 m^{-3} of soil dry weight.

Meteorological conditions (rainfall and mean air temperature) were monitored during the trial by a meteorological station sited at the experimental farm.

The field trial was arranged in a randomized block experimental design with four replicates, consisting of six plants per plot, with a density of 1.0 plant m^{-2}.

2.2.2. Plant material and management practices

Mycorrhizal (VPM) and non mycorrhizal (VPN) 4-month-old plants were manually planted in the last week of July 2011. Sprouted semi-dormant offshoots (VPO), common called "ovoli", were also planted as the control.

The irrigation was performed by the polyethylene drip tubing method, placing one pipeline for row, when water lost by evapotranspiration (ETc) reached the 50% of the available water depletion in the soil layer explored by roots, with a watering volume able to restore 100% of the water lost. The ETc was calculated by the evopotranspirometric method, using daily values of class A pan evaporation, pan coefficient equal to 0.8 (Castrignanò et al., 1985), and the crop coefficients reported by Allen et al. (1998). The irrigation season lasted from the end of July 2011 through to May 2012. Total irrigation volume applied was 4300 m^3 ha^{-1}. At the second cropping season, the regrowth of the plants was stimulated in the last week of July with a sprinkler irrigation system, while the subsequent irrigation followed the same steps as the first season.

The field experiment was conducted under low chemical inputs applied according to the nutrient availability in the soil and the expected effect of the mycorrhizal fungi, in agreement with a previous study carried out on the same soil characteristics, cropping practices and genotype (data not yet published). In the first crop season, nitrogen was applied by fertigation in small doses of 30 kg ha^{-1} of N as calcium nitrate, two months after planting, at the first appearance of heads and when the offshoots began to thin out. The same steps were then repeated after regrowth of the artichoke field and during the crop cycle of the second season. For the second year of production, a phosphorus