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The arbuscular mycorrhizal symbiosis can overcome reductions in yield and nutritional quality in greenhouse-lettuces cultivated at inappropriate growing seasons

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ABSTRACT

The association of *Lactuca sativa* L. with arbuscular mycorrhizal fungi (AMF) can benefit growth and induce the accumulation of mineral nutrients and antioxidants in leaves. Our objectives were (1) to assess to what extent growing season influence growth and nutritional quality of the green-leaf Batavia Rubia Munguía (BRM) and the red-leaf Maravilla de Verano (MV) lettuce cultivars; (2) to test if the growing season affected the effectiveness of AMF as enhancers of the nutritional quality of the aforementioned cultivars; and (3) to check if inoculation with AMF allows extending the season to cultivate these two lettuce cultivars, without a loss of plant growth or nutritional quality. Results showed that winter was the most favourable growing season for both types of lettuce but mycorrhizal plants cultivated in other seasons different from winter achieved higher or similar shoot biomass than their respective non-inoculated controls in winter. AMF induced the accumulation of Fe, proteins, carotenoids and anthocyanins in both cultivars of lettuce in winter and spring. In summer and autumn, both types of lettuce showed increased levels of anthocyanins when associated with AMF. Mycorrhizal BRM also had enhanced levels of K, sugars and ascorbate in winter and spring as well as increased amounts of Mg and Cu in winter and summer. Mycorrhizal MV had increased quantities of Cu, Zn and sugars in spring and Mn in autumn. After estimating intakes of mineral elements through the consumption of 150 g per day of fresh leaves from mycorrhizal lettuces we may affirm that there would not be toxicological risk according to the Recommended Daily Allowances (RDAs). The application of AMF could allow extending cultivation of lettuce to other periods not appropriate for producing non-mycorrhizal plants.

1. Introduction

Lettuce (*Lactuca sativa* L.) is a major food crop within the European Union and the United States of America. According to FAOSTAT (FAO Statistics Division) 2011, the total production quantity of lettuce and chicory in the European Union achieved 3,023,174 tonnes in 2010, being Italy (843,344 tonnes) and Spain (809,200 tonnes) the main producing countries. The USDA (United States Department of Agriculture) Vegetables 2011 Summary (January 2012) estimates that the total production of lettuce in USA achieved 3,889,120 tonnes in 2011. In addition, lettuce is the most used food crop for the called 'Fourth Range' of vegetables. The term originally meant fresh, cleaned, possibly chopped and mixed vegetables ready to be seasoned and eaten (Borghi, 2003). These vegetables are increasingly accepted by consumers because they are healthy and easy to prepare for eating. Moreover, lettuce exhibits healthy properties mainly due to the presence of antioxidant compounds together with high fibre content and useful amounts of some minerals in its tissues (Llorach et al., 2008; Baslam et al., 2013).

Batavia Rubia Munguía (BRM) and Maravilla de Verano (MV) are two cultivars of lettuce (*L. sativa* L. var. Capitata) extensively cultivated in greenhouses, highly commercialized and very appreciated to be consumed in salads in Spain. Both cultivars are characterized for an excellent shelf life that allows maintenance of their crispness from the time they are harvested until the time they are consumed. In European regions with mild winter, recommended sowing time for the cultivar BRM begins in January and finishes in May, being

Abbreviations: A, autumn; AMF, arbuscular mycorrhizal fungi; ASC, ascorbate; BRM, Batavia Rubia Munguía; chl, chlorophyll; CI, commercial inoculum; DM, dry matter; FW, fresh weight; MEI, mycorrhizal efficiency index; M, mycorrhizal; MV, Maravilla de Verano; NM, non-mycorrhizal; S, spring; Su, summer; TSS, total soluble sugars; W, winter.

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the respective transplantations at the end of February, March, April, May and June (CEE rules and standards, Eurogarden-S.-F.-006, Barcelona, Spain). For the cultivar MV recommended sowing time takes place in two different periods: winter (January, February and March) and summer (July, August and September) and the respective transplantations would be one month after sowing (CEE rules and standards, Eurogarden-S.-F.-006, Barcelona, Spain).

Growth and nutritional value of lettuce plants depend on environmental factors and also change during plant growth and subsequent storage. According to Glenn (1984), the interaction of day temperature and radiation was the most important factor correlated with growth. Nitrate accumulation tends to be higher in the winter than in other seasons (Burn et al., 2004) due to the reduction of nitrate reductase activity within the leaves under low light intensity and low temperatures (Riens and Heldt, 1992). In a recent work, Boo et al. (2011) concluded that at relatively low temperatures, lettuce plants have a high antioxidant and enzymatic status.

Mycorrhizal fungi colonize the roots of over 90% of plant species mostly to the mutual benefit of both plant host and fungus (Smith and Read, 2008). The most common are the arbuscular mycorrhizas (AM), which are formed by the majority of crop and horticultural plants, including lettuce. This symbiosis can benefit growth (Baslam et al., 2011a), contribute to water uptake (Ruiz-Lozano and Azcón, 1995) and improve the nutritional quality of lettuce cultivated under both optimal (Baslam et al., 2011a) and restricted water regimes (Baslam and Goicoechea, 2012). Among different species of the AMF, *Rhizophagus intraradices* (Schenck and Smith) Walker & Schüβler comb. nov. and *Funneliformis mosseae* (Nicol. and Gerd.) Walker & Schüβler comb. nov. have behaved as very effective AMF not only in taking up soil water (Marulanda et al., 2003) but also in enhancing growth and inducing the accumulation of antioxidant compounds (Baslam and Goicoechea, 2012; Baslam et al., 2011a) in leaves of several varieties and cultivars of greenhouse-grown lettuces. However, the effectiveness of AMF for improving the nutritional quality of lettuce can be affected by culture practices, such as the type of phosphorus source applied to fertilize lettuce plants (Baslam et al., 2011b). In addition, cultural practices can also affect the synthesis and accumulation of secondary metabolites interesting for the human diet (e.g. phenolic compounds) in food crops (Zhao et al., 2007).

The objectives of this study were (1) to assess to what extent different growing seasons can influence growth and nutritional quality of two types of lettuce, the green-leaf BRM and the red-leaf MV; (2) to test if the cultivation season affected the effectiveness of a commercial inoculum of AMF (a mixture of *R. intraradices* and *F. mosseae*) previously known for its ability for improving the nutritional quality of the aforementioned cultivars of lettuce; and (3) to check if inoculation with AMF allows extending the season to cultivate these two lettuce cultivars, without a loss of plant growth or nutritional quality.

2. Materials and methods

2.1. Biological material and experimental design

Batavia Rubia Munguía (BRM) (*L. sativa* L. var. Capitata) and Maravilla de Verano (MV) (*L. sativa* L. var. Capitata) were the two cultivars of lettuce chosen for this study. For each cultivar of lettuce (BRM or MV), the experiment was conducted as a factorial design with two factors and six-fold replication. The first factor had two levels: non-mycorrhizal plants (NM) and plants inoculated with AM fungi (M). The second factor included four cultivation seasons: summer (Su), autumn (A), winter (W) and spring (S).

Seeds of BRM and MV were surface sterilized by 10% bleach for 10 min and sown in a mixture of light peat and siliceous sand (1:1, v:v). Sowing times were 20th June 2009 (Su), 20th September 2009 (A), 7th January 2010 (W) and 28th March 2011 (S). When seedlings had 2–3 fully developed leaves (21 days after sowing), they were transferred to 1.5 L pots (one plant per pot and 12 pots per each type of lettuce in every season) filled with a mixture of vermiculite-siliceous sand-light peat (2.5:2.5:1, v:v:v). Peat (Floragard, Vilassar de Mar, Barcelona, Spain) had a pH of 5.2–6.0, 70–150 mg L^{-1} of nitrogen, 80–180 mg L^{-1} P_2O_5 and 140–220 mg L^{-1} K_2O and it was previously sterilized at 100 °C for 1 h on three consecutive days. In every season, at transplanting, 6 pots of each cultivar of lettuce were inoculated with the commercial inoculum AEGIS Endo Gránulo and other 6 pots were not inoculated and kept as non-mycorrhizal controls. The commercial inoculum (CI) was a mixture of *R. intraradices* (Schenck and Smith) Walker & Schüβler comb. nov. and *F. mosseae* (Nicol. and Gerd.) Walker & Schüβler comb. nov. that contained around 100 spores and other infective propagules per gram of product and was provided and commercialized by Atens (Tarragona, Spain). A total of 9.5 g of the commercial mycorrhizal formulation was added to each pot (around 950 spores). Mycorrhizal inoculum was mixed into the top 10 cm of substrate in each pot just before transplanting of seedlings in order to facilitate the early contact between AMF and growing roots of young lettuce plants.

Lettuce plants were grown in a greenhouse under glass in order to catch the maximum amount of light and achieve maximum photosynthesis. Photosynthetic photon flux (PPF) in Su, A, W and S reached, respectively, average values of 1000, 600, 400 and 700 μmol m^{-2} s^{-1}. When necessary (e.g. in some winter days), natural daylight was supplemented with irradiation from fluorescent lamps (Sylvania DECOR 183, Professional-58 W, Erlangen, Germany) that provided a minimum photosynthetic photon flux (PPF) of around 400 μmol m^{-2} s^{-1} during a 16 h photoperiod. The temperature was controlled between 25 °C and 15 °C day/night. However, daytime temperature sometimes achieved 26 °C in summer and only 21 °C in winter. Outside mean temperatures, maximum and minimal media were, respectively, 20.3, 27.8 and 13.4 °C in summer; 10.7, 16.1 and 5.8 °C in autumn; 4.5, 8.5 and 0.8 °C in winter; and 15.1, 21.3 and 8.9 °C in spring according to data collected at the station of Pamplona (ETSIA, UPNA), latitude 4738834, longitude 612067, altitude 500 m. Relative humidity (RH) always reached similar values: 40/80% day/night. All plants were fertilized once a week with 300 mL of complete Hewitt's nutrient solution with some modifications (Baslam et al., 2011a) and also received additional distilled water to maintain optimal water supply. Plants were harvested seven weeks after transplanting. Samples for analytical determinations were collected from both inner (internal zone) and outer (external zone) leaves of lettuces. Both zones were visually delimited and each one had a mean of approximately 15 leaves. The internal zone was quite close to the meristematic tip of the shoot and included light green leaves. The harvested inner leaves were located midway between the centre of the head and the outer portion. Outer leaves exhibited darker colour and larger size than inner leaves and were not compact in the lettuce head.

2.2. Growth parameters and mycorrhizal analysis

At harvest, 3 plants of each cultivar of lettuce and treatment were randomly selected for determining fresh weight (FW) of the aerial part and biomass production of both shoots and roots. Fresh samples of leaves and roots were stored at −80 °C for biochemical determinations. Dry matter (DM) of leaves and roots was determined after drying the plant material of other 3 plants at 80 °C for at least 2 days (until the weight remained constant).

Root samples of lettuce plants were cleared and stained as described by Phillips and Hayman (1970) and mycorrhizal colonization was determined by examining 1 cm root segments