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Selenium fertilization and mycorrhizal technology may interfere in enhancing bioactive compounds in edible tissues of lettuces

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) favored the accumulation of secondary metabolites in leaves of lettuces in previous studies. When fertilized with selenium (Se), mycorrhizal lettuces had more proteins, sugars and minerals than non-mycorrhizal ones. However, Se contents were lower in mycorrhizal plants suggesting a negative correlation between Se and AMF applications. The aim of the present study was to test if Se fertilization interfered with AMF technology for enhancing secondary metabolites in lettuces. Green and red-leaf lettuces were or not inoculated with AMF and received or not different selenocompounds. Flavonols and anthocyanins were non-destructively measured. At harvest, growth, water status, chlorophylls, carotenoids, phenolics and antioxidant capacity were determined. In green-leaf lettuces Se application counteracted benefits of AMF on chlorophylls and carotenoids and decreased phenolics. In red-leaf lettuces, sodium selenite positively interacted with AMF in enhancing flavonols, but imidoselenocarbamate reduced flavonols in mycorrhizal plants. No significant interaction between AMF and Se was detected for the total antioxidant capacity in leaves of both types of lettuces. The efficiency of mycorrhizal technology for improving antioxidant compounds in the edible tissues of lettuces can be modified under Se fertilization, being the interaction positive or negative depending on lettuce cultivar, antioxidant compound and chemical form of selenocompound.

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1. Introduction

Selenium (Se) is a naturally occurring micronutrient with essential biological functions for all mammalian species. Interest in compounds containing Se has increased in the past three decades, mainly due to their biological activities (Rayman, 2012). It is known to play an important role in both antioxidant defense (Ahmad et al., 2012) and immune function (Mehdi et al., 2013) and belongs to one of the most extensively studied chemopreventive and anticancer compounds (Ibáñez et al., 2011; Jayaprakash and Marshall, 2011; Lamberto et al., 2013; Moreno et al., 2012). The essentiality of Se for higher plants has not been still established but it is known that this element can help plants to cope with several environmental stresses by counteracting the negative effects caused by trace metals contamination, water deficit, UV-B radiation, salinity or extreme (high and low) temperatures on plant metabolism, physiology and growth (Feng et al., 2013).

Lettuce (*Lactuca sativa* L.) is one of the most consumed leaf vegetables in many parts of the world because it is perceived as a 'healthy' food. The healthy properties of lettuce are attributed to a large supply of antioxidant compounds (such as vitamins C and E, carotenoids, polyphenols) and fiber content (Llorach et al., 2008;

Abbreviations: AMF, arbuscular mycorrhizal fungi; ANT, anthocyanins; BRM, Batavia Rubia Munguía; chl, chlorophyll; DM, dry matter; FLAV, flavonols; FW, fresh weight; MEI, mycorrhizal efficiency index; +M, plants inoculated with mycorrhizal fungi; −M, plants not inoculated with mycorrhizal fungi; MV, Maravilla de Verano; RWC, relative water content; SeNa, sodium selenite; $SeCH_3$, imidoselenocarbamate; SeU, selenium urea; TW, turgid weight.

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Nicolle et al., 2004; Serafini et al., 2002). Other phytochemicals that contribute to both the sensory and health-promoting properties of lettuce are anthocyanins and chlorophylls (Li et al., 2010), being anthocyanins more abundant in the red than in the green varieties (Llorach et al., 2008). Moreover, the nutrient content of this vegetable includes useful amount of some minerals such as calcium and iron (Romani et al., 2002). Beyond its basic nutrition, lettuce has been the target for Se biofortification in providing daily requirement of Se (Ramos et al., 2010; Ramos et al., 2010). Supplementation of Se to lettuces can also be beneficial for plants that are undergoing environmental stresses. In a field experiment, He et al. (2004) demonstrated that the addition of selenite to lettuce plants subjected to trace metals contamination reduced the amount of Pb and Cd accumulated in plant tissues. Xue et al. (2001) found that the addition of selenate to senescing glasshouse-grown lettuce exerted an antioxidative and growth promoting effect. Se biofortification may cause changes in the synthesis of secondary metabolites that enhance plant defense against insects and herbivores and/or improve nutritional quality of crop plants (Malagoli et al., 2015). In fact, the assimilation of Se in plants can affect both sulfur and nitrogen metabolic pathways and therefore the synthesis of S-secondary compounds, N-secondary compounds or amino acids that are precursors of phenolic compounds.

Mycorrhizal fungi colonize the roots of over 80% 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, which are formed by the majority of crop and horticultural plants, including lettuce. This symbiosis can benefit growth (Baslam et al., 2011) and improve the nutritional quality of lettuce by enhancing the levels of mineral nutrients, proteins, sugars and antioxidant compounds (i.e., phenolics, ascorbate, tocopherol, carotenoids) in the edible part of this vegetable (Baslam et al., 2011, 2012, 2013).

To our knowledge there are few studies dealing with the interaction between Se fertilization and arbuscular mycorrhizal fungi (AMF) inoculation for enhancing nutritional quality of crops. Moreover, these studies, mainly focused on the absorption and accumulation of Se in host plants, reveal contradictory findings, being results highly dependent on the plant species and the chemical form of the applied selenocompound. Even when the same host plant is used (i.e., garlic), mycorrhizal inoculation can increase the Se uptake by roots (Larsen et al., 2006) or have no significant effects (Patharajan and Raaman, 2012). A recent study performed by our group (Sanmartín et al., 2014) revealed that mycorrhizal inoculation diminished or avoided the accumulation of Se in leaves of lettuces when different inorganic or organic selenocompounds were added with the nutrient solution supplied to plants. However, plants inoculated with AMF had higher contents of minerals, proteins and/or sugars than the non-mycorrhizal controls that were fertilized with Se suggesting that AMF inoculation may be adequate for growing lettuces on soils rich in Se. Therefore, we can hypothesize that the known beneficial effect of AMF on mineral uptake (Baslam et al., 2011) and photosynthesis (Sánchez-Díaz et al., 1990) of host plants was not depressed by the addition of selenocompounds, which may result in adequate levels of precursors (mainly monosaccharides) for the synthesis of secondary metabolites with antioxidant properties.

The objective of the present study was to assess to what extent the previously demonstrated effectiveness of AMF for inducing the accumulation of some antioxidant compounds (mainly produced through pathways of the secondary metabolism) in the edible part of lettuces could be affected when plants are subjected to high levels of Se in the soil. In our study, different chemicals forms of Se were added in the substrate simulating a Se biofortification program.

2. Materials and methods

2.1. Chemical and synthetic methods

The selenocompounds applied in the present study were the same previously used by our group with lettuce plants (Sanmartín et al., 2014): sodium selenite (SeNa), methyl *N*,*N*′-bis(4-fluorophenyloxycarbonyl) imidoselenocarbamate ($SeCH_3$) and *N*′,*N*′′′-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl) selenourea]·4HCl (SeU).

Sodium selenite was used because there is strong evidence that selenite and phosphate (Pi) share similar uptake mechanisms and transporters (Zhang et al., 2014). In the associations between AMF and plant roots there are several Pi transporters involved: plant-specific Pi transporters in root hairs and epidermal cells, fungal-specific Pi transporters in the extraradical hyphae and symbiosis-specific host Pi transporters that transfer Pi from fungal arbuscules to plant cortical cells (Liu et al., 2015). The organoselenic derivatives ($SeCH_3$ and SeU) contained structural fragments potentially useful for the biological action pursue, such as: (1) certain degree of molecular symmetry; (2) combination of chemical groups and bonds from different degree of structural rigidity that can confer enough flexibility to molecules; (3) variable contents of selenium and nitrogen elements and different kind of bond for these elements. Both organocompounds have distinct types of halogens; (4) include methylseleno moiety, which mimetic selenomethionine, placed on a group that allow his release by hydrolysis. It is remarkable that methyls eleno moiety is critical for the biological activity acting through different mechanism (Fernandes et al., 2012); (5) a compound possess the selenourea scaffold, structural analogue of urea, that it is one of the most studied fertilizers and the other compound possess the more polar carbamate group. Se biofortification is most effective when organic Se is supplied to crop plants (Malagoli et al., 2015).

Sodium selenite 99% Na_2SeO_3 was purchased from Sigma–Aldrich, St. Louis, MO. Methyl *N*,*N*′-bis(4-fluorophenyloxycarbonyl) imidoselenocarbamate ($SeCH_3$) (Fig. 1) was prepared from methylimidoselenocarbamate hydroiodide and 4-fluorophenyl chloroformate and *N*′,*N*′′′-(diselanediyldibenzene-4,1-diyl)bis[1-(4-chlorophenyl)selenourea]·4HCl (SeU) (Fig. 2) was synthesized by reaction between 4-chlorophenylisoselenocyanate and 4,4′-diselanediylaniline as previously reported by us (Sanmartín et al., 2014).

2.2. Biological material and experimental design

The green-leaf lettuce Batavia Rubia Munguía (*L. sativa* L. var. Capitata) (BRM) and the red-leaf lettuce Maravilla de Verano (*L. sativa* L. var. Capitata) (MV) were used in this study. Seeds of BRM and MV were surface sterilized by 10% bleach for 10 min and sown in a mixture of peat and sand (1:1, v:v). When seedlings had 2–3 fully developed leaves, they were transferred to 1.5 L pots (one plant per pot, 32 pots with BRM and 32 pots with MV thus making a total of 64 pots) filled with a mixture of vermiculite-sand-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. At transplanting, 16 pots with BRM and 16 pots with MV were inoculated with 13 g of the commercial inoculum AEGIS Endo Gránulo (+M plants). The application of this commercial inoculum was found to improve the quality of greenhouse-lettuce in previous studies (Baslam et al., 2011; Baslam et al., 2011) and its effectiveness for improving growth and inducing the accumulation of antioxidant compounds in lettuce plants was previously compared with that of *Glomus fasciculatum* (Taxter *sensu* Gerd.) Gerd. and Trappe come from a pot culture with

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