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Effects of foliar nitrogen fertilization on the phenolic, mineral, and amino acid composition of escarole (*Cichorium endivia* L. var. latifolium)

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ABSTRACT

Nitrogen has a pivotal role in determining the quality of horticultural products, and foliar fertilization strategies could achieve higher nutrient use efficiencies while reducing environmental impacts and potentially enhancing consumer health benefits. Escarole (*Cichorium endivia* L. var. latifolium) plants were grown in nutrient solution, either complete or without N, and sprayed with different concentrations of foliar urea (0, 1, 5, and 10 g L^{-1}). Total protein in the leaves was increased by the elevated concentrations of urea but the levels of total phenolics and total soluble sugars were lower. The contents of minerals in plants not receiving N in the nutrient solution were, in general, significantly increased by urea applications, but those of Cu and Zn were decreased. Additionally, the amino acids concentrations were boosted after urea application, whatever the composition of the irrigation solution, arginine, serine, and alanine being the most abundant amino acids. In conclusion, foliar N fertilization was an effective strategy to enhance the nutritional properties.

1. Introduction

Nowadays, a large proportion of people worldwide consume fruits and vegetables in insufficient quantities; an increase in the intake of these foods can be an effective strategy to improve the cognitive health of older adults (Miller et al., 2017). Foods have now widely assumed the status of "functional foods" and phytochemicals act as antioxidants and scavengers of free radicals, block enzymes that potentiate cancer, prevent the accumulation of carcinogens, or act as elicitors that help to produce enzymes that interfere with the reproduction of malignant tumors (Kaur and Kapoor, 2001).

Phenolics are carbon-based chemical compounds that play significant roles (for instance, in physiological and ecological processes) in plants, in constitutive and induced defense against herbivores, weeds, and pathogens and in structural support (Jones and Hartley, 1999). Moreover, phenolics are of significant importance in the organoleptic, nutritional, and commercial properties of horticultural products, through sensory properties, in addition to their preventative and treatment potential in combating cancer and age-related disorders (Wahle et al., 2010). Escarole (*Cichorium endivia* L. var. latifolium) is an important leafy vegetable with increasing interest worldwide because of its high nutritive value and healthy properties (Llorach et al., 2008). It is increasing in popularity in mixed ready-to-eat salads, being appreciated for its distinctive crunchy texture and mildly bitter taste (D'Antuono et al., 2016).

Modern agriculture has to increase yields and product quality while reducing environmental impacts. Thus, in our current scenario of climate drift, improved crop management is required to optimize natural resources, providing new solutions to nourish mankind. Nitrogen (N) fertilizer is one of the most important factors that boost plant growth, but public concern has led to legislation to reduce nitrate concentrations in water resources (Suárez-Rey et al., 2016). Little more than half of the total N applied in agriculture as inorganic fertilizers is taken up by crops; the remainder contributes to pollution of ground waters, with ecological and human health effects (Campiglia et al., 2014). Therefore, improvement of N uptake and application efficiency has the potential to reduce N contamination. The method of N application (to soil or leaves) has a significant influence on crop product quality, through effects on growth and storage of N (Habib et al., 1993) but also on the oxidative metabolism, through production of active oxygen species (del Amor et al., 2009).

Foliar application of fertilizers has important benefits for crop production, supplying nutrients when soil conditions limit root uptake and achieving higher application efficiency as the fertilizers are

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required in much lower quantities, and can supply at least part of the N required to sustain growth (Nicoulaud and Bloom, 1996). However, the effectiveness of foliar N applications depends on the environmental conditions (del Amor and Cuadra-Crespo, 2011). Urea is absorbed rapidly by the leaves of most crop plants (Reickenberg and Pritts, 1996), being an effective strategy to reduce the amount of N lost to the environment.

Nitrogen has a pivotal role, controlling both the yield and quality of vegetables, but it also influences the biosynthesis of secondary metabolites (Aires et al., 2006). To the best of our knowledge, there is no literature available about the nutritional composition of escarole in relation to foliar N fertilization strategies. Thus, in the context of environmental sustainability and food quality, the aim of the present study was the evaluation of the mineral composition, total phenolics and carbohydrates, and amino acid profile of this important crop, when the N supply was withdrawn from the roots and applied to the leaves. The results from this study could help to optimize quality without compromising the environment.

2. Material and methods

2.1. Plant material and growth conditions

Escarole (*Cichorium endivia* L. var. latifolium) seeds were germinated on a mixture of peat and perlite (3:1). Seedlings were selected for uniformity after 25 days and transplanted to 15-L round black containers containing a modified Hoagland solution (control) which was prepared with the following salts, in mM: 4.52 $Ca(NO_3)_2{\cdot}4\ H_2O$, 2.93 KNO_3, 2 $MgSO_4{\cdot}7\ H_2O$, 1.48 K_2SO_4, 1 KH_2PO_4, and micronutrients. Conductivity and pH were 2.5 dS m^{-1} and 6.0 respectively. This kind of containers had a hole in the center of the cover (place where escarole plants were transplanted) and the plants were supported by using pieces of polyurethane foam. Besides, the solution was aerated continuously through independent microtube connected to an air compressor. The plants were raised under these conditions for 14 days, before the treatments started. The experiment was carried out in a climate chamber, designed by our department specifically for plant research proposes (del Amor et al., 2010), with fully-controlled environmental conditions: 65% relative humidity, 16/8 h day/night (22/15 °C), and a photosynthetically-active radiation (PAR) of 250 µmol m^{-2} s^{-1} provided by a combination of fluorescent lamps (TL-D Master reflex 830 and 840, Koninklijke Philips Electronics N.V., The Netherlands) and high-pressure sodium lamps (Son-T Agro, Philips). After the acclimation period, half of the plants were supplied for 15 days, with a nutrient solution without N (2.32 $MgSO_4{\cdot}7\ H_2O$, 2.56 K_2SO_4, 3 KH_2PO_4, 5.5 $CaSO_4{\cdot}2\ H_2O$, 0.016 KOH). For this solution, conductivity and pH were 2.6 dS cm^{-1} and 6.2 respectively. Thus, the treatments consisted of two nutrient solutions combined with four foliar urea concentrations: 0 (distilled water), 1, 5, and 10 g L^{-1}. Tween 20 (0.5%) was used as the surfactant for all treatments. The plants were sprayed completely and homogeneously every seven days. At the same time, the nutrient solution in each container was renewed completely to avoid nutrient imbalances and increases in electrical conductivity. The experiment lasted a month.

2.2. Mineral content

The youngest full-sized leaves of each plant (four plants per treatments) were dried for 72 h at 65 °C in a heater. The cations were extracted from ground material (0.1 g) by acid digestion, using an ETHOS ONE microwave digestion system (Milestone Inc., Shelton, CT, USA). The Ca, K, Mg, B, Cu, Fe, Mn, P, and Zn concentrations in the dry matter of the leaves were analyzed with an inductively-coupled plasma (ICP) spectrometer (Varian Vista MPX, Palo Alto, CA, USA). Analyses were run in four replicates.

2.3. Total protein

The total protein was analyzed in the dry matter of youngest full-sized leaves (after at least 72 h at 65 °C) using a combustion nitrogen/protein determinator (LECO FP-528, Leco Corporation, St. Joseph, MI, USA). Protein analyses were carried out in four replicates.

2.4. Total phenolic compounds

The total phenolic compounds were extracted from 0.5 g of the youngest full-sized leaves (frozen at −80 °C) with 5 mL of 80% acetone. The homogenate was centrifuged at 10,000 rpm at 4 °C, for 10 min. Folin–Ciocalteu reagent was used, diluted with Milli-Q water (1:10): 1 mL of the diluted reagent was mixed with 100 µL of supernatant and 2 mL of Milli-Q water, and 5 mL of sodium carbonate (20%) were then added. The mixture was kept for 30 min in the dark. The absorbance was measured at 765 nm, according to the methodology of Kähkönen et al. (1999). The total phenolic content was expressed as gallic acid equivalents, in mg g^{-1} dry weight. Analyses were carried out in four replicates.

2.5. Total soluble sugars

Soluble sugars were extracted according to Walker et al. (2008), by incubating 40 mg of lyophilized leaf tissue (youngest full-sized leaves) twice in 5 mL of 60% ethanol, for 30 min each time, at 35 °C. Each extract was centrifuged at 3500 × g for 10 min, at 20 °C, and the two supernatants were combined. Chloroform (5 mL) was added and the mixture shaken before centrifugation at 2700 × g for 10 min, at 20 °C. The upper, colorless layer (20% ethanol) was diluted four-fold with absolute ethanol, to produce an extract in 80% ethanol for the measurement of soluble sugars according to Buysse and Merckx (1993). Soluble sugars analyses were run for four replicates.

2.6. Free amino acids

The free amino acids were extracted from the youngest full-sized leaves (frozen at −80 °C): the sap was extracted, after vortexing at 5,000 rpm (10 min, 4 °C), and analyzed by the AccQ•Tag-ultra ultra-performance liquid chromatography (UPLC) method (Waters, UPLC Amino Acid Analysis Solution, 2006). For derivatization, 70 µL of borate buffer were added to 10 µL of sap and 20 µL of reagent solution. The reaction mixture was mixed instantly and heated at 55 °C for 10 min. After cooling, an aliquot of the reaction mixture was used for injection. The column was an Acquity BEH C18 1.7 µm, 2.1 mm × 100 mm (Waters), and the wavelengths were set at 266 nm (excitation) and 473 nm (emission). The solvent system consisted of two eluents: (A) AccQ•Tag-ultra eluent A concentrate (5%, v/v) and water (95%, v/v); (B) AccQ•Tag-ultra eluent B. The following elution gradient procedure was used for the analysis: 0–0.54 min, 99.9% A–0.1% B; 5.74 min, 90.9% A–9.1% B; 7.74 min, 78.8% A–21.2% B; 8.04 min, 40.4% A–59.6% B; 8.05–8.64 min, 10% A–90% B; 8.73–10 min, 99.9% A–0.1% B. The injection volume was 1 µL, with a flow rate of 0.7 mL min^{-1}. The temperature of the column was maintained at 55 °C. External standards (Thermo Scientific) were used for the quantification of the amino acids, and Empower 2 (Waters) software for data acquisition and processing. Free amino acids analyses were carried out in four replicates.

2.7. Statistical analysis

The data were tested for homogeneity of variance and normality of distribution. Analysis of variance (ANOVA) was performed and means were separated by Duncan's multiple range test at $P \leq 0.05$, using the Statgraphics Centurion® XVI statistical package (Statpoint Technologies, Inc.). A total of eight treatments were studied, involving