Available online at www.sciencedirect.com
ScienceDirect
Scientia Horticulturae 110 (2006) 21–29
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Effect of iron supply and nitrogen form on growth, nutritional status and ferric reducing activity of spinach in nutrient solution culture

A. Assimakopoulou *

Laboratory of Non Parasitic diseases, Benaki Phytopathological Institute, 8 St. Delta Str, GR-145 61 Kifissia, Attiki, Greece

Received 26 February 2005; received in revised form 20 November 2005; accepted 13 June 2006

Abstract

This study was carried out in order to give some information that could improve spinach nutritional status and productivity. In this paper, the effect of two N forms (N was added either as 100% nitrate or as 80% nitrate and 20% ammonium) and three Fe levels (0 μM Fe; 20 μM FeEDDHA; 3 μM FeEDDHA + 10 mM $NaHCO_3$) on the growth, chlorosis symptoms and shoot nutrient element accumulation was studied in spinach plants (var. Viroflay), grown in hydroponics; six treatments and three harvests (at about 20 days interval each, until plants reached their commercial size) were applied in total. The results indicated that under conditions of Fe sufficiency (20 μM Fe), mixed N nutrition induced higher production of dry matter as well as improved Fe, Mn and Zn plant nutritional status. In plants grown under Fe deprivation (0 μM Fe), shoot Fe concentration was not significantly affected by the N form until the end of the experiment despite mixed N nutrition induced higher dry matter production up to harvest 2; plants grown under Fe deprivation and with mixed N nutrition presented also higher shoot Mn and Zn concentration. Under conditions of high concentration of bicarbonates and low level of Fe (3 μM Fe + 10 mM $NaHCO_3$), the N form had not a significant influence on total dry matter production whereas shoot Fe and Mn accumulation in 100% NO_3-fed plants was found to be significantly reduced compared to mixed N nutrition; regardless of the N form, those plants presented the least dry matter production, highest intensity of leaf chlorosis as well as highest root ferric reducing activity compared to plants grown under Fe deprivation.

Keywords: Fe-deficiency; Lime-induced chlorosis; N form; Nutrient elements interactions; Spinach

1. Introduction

It is well known that nutrient availability to people is primarily determined by the output of foods produced by agricultural systems. Importantly, plant foods provide most of the nutrients that feed the developing world; agriculture must change in ways that closely link food production to human health and nutritional requirements.

Specifically, it is also well known that nutrient elements in the leaves of spinach are always important to human health (Welch, 2002); spinach as a dietetic nutrient has long been the object of many investigations.

Unexceptionably, nitrogen plays a pivotal role in the inorganic nutrition of plants and hence in determining growth. The form of N supply, to a great extent, controls the uptake ratio of cations and anions and thus, influences dry matter production and root rhizosphere and apoplastic pH (Mengel, 1995; Marschner, 1997). It has repeatedly been reported that micronutrients interactions with N occur frequently due to change in the nutrient solution pH with the addition and uptake of NH_4-N and NO_3-N; when more NO_3-N is applied, rhizosphere pH increases whereas when more NH_4-N is absorbed, rhizosphere pH decreases. Specifically, Fe nutrition of plants can be significantly affected by N form because of the aforementioned changes in the rhizosphere and apoplastic pH and the uptake ratio of cations and anions (Mengel et al., 1994).

Fe chlorosis as a matter of simple deficiency is seldom the case because Fe availability is often restricted by the limited solubility of Fe oxides in aerobic environments or by elevated concentration of nitrate and bicarbonate on calcareous soils (Mengel, 1995). Both Fe level and N source in plants growth medium are involved in multiple interactions with other elements; the effects of interactions are expressed in different ways, including uptake phenomena and biochemical reactions (positive and negative synergisms, competition, protection, etc.); some interactions are the result of Fe deficiency and

* Present address: District Laboratory of Advisory Service and Fertilizer Analysis, GR-204 00 Xylokastro, Greece. Tel.: +30 2743 0 22428; fax: +30 2743 0 22419.
E-mail address: dalakl@otenet.gr.

0304-4238/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2006.06.010

others are the cause of Fe deficiency, directly or indirectly; for example, it has been observed that ammonium-N has induced Fe deficiency because of the increased uptake of P (Wallace et al., 1992). In crops whose commercial yields are the leaves, such as lettuce, spinach, endive, cabbage, etc., a great number of studies have been done on the influence of N fertilization (rate and form) on yield, nitrate accumulation and ion composition (Cantliffe, 1972a,b; Barker et al., 1974; Wang and Tadashi, 1997; Santamaria and Elia, 1997; Wang and Li, 2004; Simonne et al., 2001). Evaluating the effect of nitrogen form under different iron levels in the growth medium on nutrient element accumulation in leafy vegetables could provide some useful information for improving their nutritional status. In this work, we started studying the impact of N form under varied Fe levels in the growth medium on growth characteristics, mineral nutrition and physiological responses in spinach.

2. Materials and methods

2.1. Plant culture

In November 2002, after soaking in 1 mM $CaSO_4$ overnight, seeds of the smoothly leaved cultivar Viroflay of *Spinacia oleraceae* L. were germinated and grown in sand culture for 10 days, receiving half strength the Long Ashton nutrient solution for macronutrients and full for micronutrients; in that precultured period 10 μM FeEDDHA were added to the nutrient solution. When the mean initial fresh weight of plants was 175.0 mg, 144 plants were selected in total to start the experiment. The seedlings were transplanted to individual 4 L plastic pots (one seedling per pot), filled with medium grade silica sand and placed in a glasshouse without supplementary heating and lighting; the mean temperature from 22 to 30 November 2002 was 19.6 °C, from 1 to 31 December 2002 11.6 °C and from 1 to 22 January 2003 14.3 °C. The pots were arranged in a completely randomised block factorial design with eight replicates, three Fe levels, two N ratios, at three growth stages corresponding to three harvests. Six treatments (I–VI) were applied in total; the six relevant nutrient solutions consisted a combination of three Fe levels (0 μM Fe, 20 μM FeEDDHA, 3 μM FeEDDHA Fe + 10 mM $NaHCO_3$ + 0.5 g $CaCO_3$ L^{-1} nutrient solution) and two N ratios (N was added either as nitrate, 100% NO_3-N, or as nitrate and ammonium with a ratio 80% NO_3-N:20% NH_4-N, but the same total N concentration). Each plant was irrigated three times daily with 0.08 L of the modified Long Ashton nutrient solution. In the treatments (I, III, V) with 100% NO_3-N (14 mM NO_3-N), the nutrient solution consisted of: 5 mM $Ca(NO_3)_2$, 4 mM KNO_3, 1.3 mM $MgSO_4$, 2 mM KH_2PO_4, whereas in the treatments (II, IV, VI) with 80% NO_3-N:20% NH_4-N (11.2 mM NO_3-N:2.8 mM NH_4-N) the nutrient solution consisted of: 5 mM $Ca(NO_3)_2$, 1.2 mM KNO_3, 1.3 mM $MgSO_4$, 2 mM KH_2PO_4, 0.9 mM $(NH_4)_2SO_4$, 1 mM $NH_4H_2PO_4$, 2.8 mM KCl (Zornoza and Gonzalez, 1998). The concentration of micronutrients was: 100 μM NaCl, 30 μM H_3BO_3, 10 μM $MnSO_4$, 2 μM $ZnSO_4$, 1 μM $CuSO_4$, 0.5 μM $(NH_4)_6Mo_7O_{24}$. Fe concentration in the nutrient solution of treatments I, II was: 0 μM; in treatments III, IV: 20 μM and in treatments V, VI: 3 μM Fe plus 10 mM $NaHCO_3$ and 0.5 g $CaCO_3$ L^{-1} of nutrient solution. The nutrient solution of the treatments V and VI refers an imitation effort of calcareous soils solutions. The nutrient solution pH of the treatments with 0 or 20 μM Fe-EDDHA was buffered with 1 M NaOH to 6.00 whereas that of treatments with 3 μM Fe + 10 mM $NaHCO_3$ + 0.5 g $CaCO_3$ L^{-1} to 7.50.

Plants were harvested on days: 18 (harvest 1), 42 (harvest 2) and 61 (harvest 3) from the beginning of the treatments, and separated to the shoot (upper plant) and root; the root system was washed carefully, three times with deionised water. The fresh weights of the upper plant and root were taken at each harvest. Then, the plant material was dried to constant weight in a forced draught air oven at 80 °C, weighed, wet-ashed (Kjeldahl method) and dry-ashed in a furnace at 550 °C. The concentration of N and P was determined by using the indophenol-blue and molybdenum-blue method in the wet digest, respectively; K was measured with flamephotometry and Ca, Mg, Fe, Mn, Zn, Cu using a Varian A220 atomic absorption spectrometer, in the dry digest.

2.2. Chlorosis

The mean value of the chlorosis score of every pair of leaves of each experimental plant was recorded at each harvest using a one to five rating scale (1 = normal green leaves; 5 = severe chlorosis with necrotic spots) (Wei et al., 1994).

2.3. Root ferric chelate reductase activity

At the second and third harvest, the ferric chelate reductase activity (FC-R) of the roots was determined with total root systems of intact plants according to the protocol described by Romera et al. (1991).

2.4. Statistics

Each value represents the average of eight replicates except that of ferric chelate reducing activity of the roots (four replicates). Significant differences in mean values between treatments were evaluated by the ANOVA, LSD test at $P < 0.05$.

3. Results

3.1. Chlorosis score

Chlorosis symptoms appeared first on the seventh day after the beginning of the treatments, in the young leaves of plants grown with high bicarbonates and N as 80% NO_3-N:20% NH_4-N whereas plants grown with high bicarbonates and N as 100% NO_3-N showed the highest score at the end of the experiment. At harvest 3, which was coincident with the end of the experiment, there were some plants grown without Fe (0 mM Fe) with whatever N form, with no visual symptoms of Fe deficiency; as regards the main effect of Fe levels, the mean chlorosis score of plants grown without Fe was 1.20, that of plants grown with high