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Remediation of Iron Chlorosis by the Addition of Fe-*o,o*-EDDHA in the Nutrient Solution Applied to Soilless Culture

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Abstract. **The aim of this study was to evaluate the remediation of ferric chlorosis using by iron (Fe)-*o,o*-EDDHA in fertigation of soilless crops compared with Fe-EDTA (ethylene diamine tetra acetic acid) and its effects on production. Two separate greenhouse experiments were conducted in slab or bag cultures using the tomato (*Lycopersicon esculentum* Mill. cv. Daniela) and green bean crops (*Phaseolus vulgaris* L. cv. Maite) in Almería (southeast Spain). The crops were subjected to the following experimental setup: 1) At first phase, all plants were treated with a standard nutrient solution and Fe was supplied as Fe-EDTA. 2) No Fe was supplied in the nutrient solution to bean crops 46 days after transplanting. For tomato plants, this element was eliminated from the nutrient solution since 102 days after transplanting. In this phase, Fe-EDTA was supplied to the control plants (T1). This phase was ended when signs of ferric chlorosis appeared on the leaves. 3) The ferric chlorosis was remediated with either Fe-EDTA (T2) or Fe-*o,o*-EDDHA (T3). The T4 group did not receive any supplements. The total tomato and bean production was improved after the Fe deficiency had been corrected by either EDTA and Fe *o,o*-EDDHA supplements in the fertigation of these crops. The synthetic Fe *o,o*-EDDHA chelate alleviated Fe deficiency by increasing the amount of iron in the rhizosphere and its supply to the leaves and petioles. Consequently, the decrease in tomato and bean production resulting from ferric chlorosis could be prevented. As a conclusion, the remediation of ferric chlorosis through fertigation with Fe *o,o*-EDDHA is as effective as the use of traditional Fe-EDTA.**

The use of synthetic iron (Fe) chelates in fertigation is the most common method to alleviate iron deficiency in crops. Several factors that determine the effectiveness of Fe chelates have been described such as the dosage applied and how theses crops are managed (García-Marco et al., 2006). Ethylene diamine tetra acetic acid (EDTA) is among the most commonly used chelating agents in southeastern Spain. However, the most effective chelating agent is actually diamino-di-(ortho-hydroxy phenyl acetic) acid (*o,o*-EDDHA) (Lucena, 2006), because the final amount of dissolved Fe obtained from Fe *o,o*-EDDHA is greater than that from Fe-EDTA (García-Marco et al., 2006), and the dissolved Fe obtained can be maintained in solution over a wide range of pH values (Alcañiz et al., 2004). However, as a result of the high cost, only cash crops are treated with these Fe chelates (Chen and Barak, 1982). Although several researchers have investigated ferric chlorosis and ways to remediate Fe deficiency in crops grown in soil (Marschner et al., 1986; Mengel, 1995) and in soilless crops, few have compared the effects of various synthetic Fe chelates available in the market (Assimakopoulou, 2006; Hernández-Apaolaza, 2007; Lucena and Chaney, 2007).

The total area of soilless crops in southeast Spain today is ≈5000 ha, half of which uses rockwool as the growing medium, whereas the other half uses perlite, sand, coir, and other minor soilless systems (Mazuela et al., 2005).

The aim of this study was to evaluate the effectiveness of Fe *o,o*-EDDHA in alleviating ferric chlorosis compared with EDTA in a soilless crop and its effects over production.

Materials and Methods

Two separate greenhouse experiments were conducted in slab or bag cultures using the tomato (*Lycopersicon esculentum* Mill. cv. Daniela) and green bean (*Phaseolus vulgaris* L. cv. Maite) crops in Almería (southeast Spain). Commercially available Grodan® Med. rockwool and Otavi® Ibérica perlite were used in slab and bag cultures for both the tomato and green bean crops. Tomato and green bean seeds were sown on 21 Sept. and transplanted on the 1 and 7 Oct. 2006, respectively. Plant density was two per square meter (six plants per bag or slab). Fertigation was applied independently for each treatment with a localized irrigation system. pH, electric conductivity (EC), individual concentration of each nutrient, time and frequency of nutrient solution application were automatically adjusted depending on the following factors: the developmental stage of plants, the physical and physical–chemical properties of each growing medium, climatic conditions at the real time (particularly irradiation), and the drainage parameters (Salas and Urrestarazu, 2001). Local plant management was performed for each crop (Urrestarazu et al., 2005). Volume, pH, EC, and chemical analyses of drainage (data not shown, except those for Fe application) were performed weekly. pH measurements were made using a pH meter (Crison model 2000, Crison Instruments, Alella, Spain). Except for the addition of Fe chelates used, a standard nutrient solution similar that reported by Sonneveld and Straver (1994) was used.

The crops were subjected to the following experimental setup:

1. At first phase, all plants were treated with a standard nutrient solution and Fe-EDTA was supplied.
2. No Fe was supplied in the nutrient solution to the bean crops 46 d after sowing. For the tomato plants, this element was eliminated from the nutrient solution 102 d after transplanting. In this phase, Fe-EDTA was supplied to the control plants (T1). This phase (phase 2) was ended when signs of ferric chlorosis appeared on the leaves (Figs. 1 and 2).
3. Ferric chlorosis was remediated with either Fe-EDTA (treatment 2, T2) or Fe *o,o*-EDDHA [treatment 3 (T3)]. Treatment 4 (T4) group did not receive any supplements. The days after sowing 116 and 46 to tomato and green been were renewed the nutrient solution, respectively.
4. The control plants (T1) were supplied with Fe-EDTA from the beginning to the end of the test.

The Fe concentration in the leaves, drainage, and sap fluids (from leaves without petiole) were determined directly after digestion according to Benton et al. (1996). The measurement was performed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using a Spectroflame model ICP-D (SPECTRO Analytical Instruments, Kleve, Germany). All measurements were

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Fig. 1. Iron (Fe) deficiency (central image) and a normal Fe status in bean plants (right) at the end of phase 2.

Fig. 2. Iron (Fe) deficiency (left) and a normal Fe status in tomato plants (right) at the end of phase 2.

performed in triplicate. Each experiment was conducted using the randomized complete block design with four replicates. Twelve plants were assigned to each experimental unit. The least significant difference test was used to compare the significance of the differences in the treatment means obtained. The experimental designs and data analyses were based on the procedure described by Little and Hills (1987). The Stagraphics Plus 4.1 statistical package was used to process the data (Statistical Graphics Corp., 1999).

Results and Discussion

Fe is present in drainage fluids of the different treatments for both crops (Table 1). The evaluation of Fe content in drainage fluids is important because these values can be used as a control parameter of fertigation (Urrestarazu et al., 2005). During the iron deficiency phase (phase 2), the Fe concentration in drainages of T2, T3, and T4 was significantly lower than the one of T1 drainage. When Fe was applied to alleviate ferric chlorosis, the Fe content detected in the drainage fluids from T4 (no Fe provided) was much lower than that found in the drainage fluids from both T2 (treatment with Fe-EDTA) and T3 (treatment with Fe *o,o*-EDDHA). When EDTA and Fe *o,o*-EDDHA were applied, the Fe content in the drainage fluids reached levels comparable to those found in the drainage fluids from T1.

The Fe concentration levels in the leaves (Table 2) are within the range of the reference values known for tomato and green bean crops (Benton et al., 1996; Roorda and Smilde, 1981). During the Fe deficiency period, a significant decrease in the Fe content in the leaves was observed (Figs. 1 and 2), although the values were still within the normal ranges for both crops. After the addition of Fe as a nutrient supplement, the Fe content in the leaves reached levels similar to those found in the drainage fluids.

The Fe concentration in the sap fluid was similar to that found in the leaves, and no significant differences were found in beans during phase 2 (Table 3).

Taken together, the results show that Fe *o,o*-EDDHA is as effective as the more commonly used Fe-EDTA in alleviating ferric chlorosis. However, because Fe *o,o*-EDDHA

Table 1. The effect of treatments on mean iron (Fe) concentration in drainage in different phases of crops (µmol·L^{-1})

	Treatment				LSD		
Phase	T1	T2	T3	T4	0.05	0.01	0.001
Tomato							
Provoked deficiency							
Average values	43.04		← 14.68 →		6.80	9.67	14.00
Remediated deficiency	33.81	25.01	32.08	24.10	7.39		
Green bean							
Provoked deficiency							
Average values	62.79		← 40.32 →		19.75		
Remediated deficiency	128.12	86.31	90.75	72.92	10.05		

T1 = Fe as EDTA all cycle; T2 = T1 (phase 1) + provoked Fe deficiency and remediated Fe deficiency with Fe-EDTA; T3 = T2 but remediated Fe deficiency with Fe-*o,o*-EDDHA; T4 = T2 o T3 without Fe deficiency remediation.
LSD = least significant difference.

Table 2. The effect of treatments on mean leaf concentration iron (Fe) (mmol·kg^{-1})

			Treatment				
Accumulated days		Phases	T1	T2	T3	T4	$LSD_{0.05}$
Tomato	0	Sowing					
	102	Provoked Fe deficiency					
	114	Average values	4.38		← 2.59 →		1.75
	116	Remediated Fe deficiency					
	155		3.88	4.21	4.06	3.21	1.38
Fe normal contents reference values				1.80–7.00[z]			
				1.07–5.37[y]			
Green bean	0	Sowing					
	46	Provoked Fe deficiency					
	81	Average values	4.21		← 2.97 →		1.24
	93	Remediated Fe deficiency					
	120		3.18	3.28	2.07	1.05	1.02
Fe normal contents reference values				0.90–7.16[y]			

T1 = Fe as EDTA all cycle; T2 = T1 (phase 1) + provoked Fe deficiency and remediated Fe deficiency with Fe-EDTA; T3 = T2 but remediated Fe deficiency with Fe-*o,o*-EDDHA; T4 = T2 o T3 without Fe deficiency remediation.
[z]Roorda and Smilde (1981).
[y]Benton et al., (1996).
LSD = least significant difference.

Table 3. The effect of treatments on sap content iron (Fe) (µmol·kg^{-1} fresh weight)

			Treatment				
Accumulated days		Phases	T1	T2	T3	T4	$LSD_{0.05}$
Tomato	0	Sowing					
	102	Provoked Fe deficiency					
	114	Average values	108		← 47 →		46
	116	Remediated Fe deficiency					
	155		123	181	210	133	NS
Fe normal contents reference values				27–36[z]			
Green bean	0	Sowing					
	46	Provoked Fe deficiency					
	81	Average values	158		← 212 →		NS
	93	Remediated Fe deficiency					
	120		216	213	218	173	38

T1 = Fe as EDTA all cycle; T2 = T1 (phase 1) + provoked Fe deficiency and remediated Fe deficiency with Fe-EDTA; T3 = T2 but remediated Fe deficiency with Fe-*o,o*-EDDHA; T4 = T2 o T3 without Fe deficiency remediation.
[z]Roorda and Smilde (1981).
[NS]Nonsignificant.

Table 4. The effect of treatments on crops yield ($kg \cdot m^{-2}$)

	Tomato		
	Total	Early yield	Green bean
T1	7.03	2.35	3.71
T2	6.15	1.93	2.67
T3	6.25	1.99	2.81
T4	5.84	2.06	2.53
$LSD_{0.05}$	0.99	NS	1.18

T1 = Fe as EDTA all cycle; T2 = T1 (phase 1) + provoked Fe deficiency and remediated Fe deficiency with Fe-EDTA; T3 = T2 but remediated Fe deficiency with Fe-*o,o*-EDDHA; T4 = T2 o T3 without Fe deficiency remediation.
NSNonsignificant.

(Alcañiz et al., 2004) remains active for a longer period of time (Lucena, 2006), it is can be considered a more useful supplement in nutrient solution for soilless crops.

The early tomato production shows no significant differences (Table 4). The total tomato and bean production was increased after the remediation of Fe deficiency in the crops by either Fe-EDTA or Fe *o,o*-EDDHA chelate alleviates Fe deficiency by increasing the amount of Fe in the rhizosphere and its supply to the leaves and petioles. Consequently, the decrease in tomato and bean production resulting from ferric chlorosis can be prevented. As a conclusion, the remediation of ferric chlorosis through fertigation with Fe *o,o*-EDDHA is as effective as the use of traditional EDTA.

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