Changes of Morphological and Physiological Responses to Indirect Iron Deficiency of Two Apiaceae Species

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Abstract: The effect of iron deficiency (bicarbonate induced) on plant morphology and growth parameters were addressed in two apiaceae species grown in continuously aerated nutrient solution with the absence or the presence of Fe with the addition of bicarbonate during one month. Growth parameters, iron statut, chlorophyll and carotenoid content and rhizosphere acidification were studied, along with zinc and copper concentration. Our results showed a high chlorosis index and a significant decrease of chlorophyll content in both species but more marked in *P. crispum*. In addition, Fe deficiency restricted significantly the plant biomass production as well as leaves number and shoot length. A reduction in iron uptake and a variability in zinc and copper accumulation were also recorded. Moreover, a capacity of root acidification due to a noticeable proton release rate, accompanied by root development were observed in both species but more pronounced in *A. graveolens*. These findings suggest that the latter seems to be less sensitive to the stress than *P. crispum*.

Keywords: *Petroselinum crispum*, *Apium graveolens*, iron deficiency, plant morphology and growth parameters.

1. INTRODUCTION

Iron is essential for both plant growth and crop yields. Due to the insoluble nature of its chemical forms, ferric iron is not readily available for plants and approximately 30% of the arable soils are iron deficient on Earth. Consequently, iron deficiency is a major nutritional disorder in crops growing on calcareous soils. To uptake iron, plants develop several mechanisms classified in two strategies; (i) Characteristic of the dicotyledonous and non Poaceae species based on the rhizosphere acidification by the activation of a plasma membrane in roots and Fe^{3+} reduction by a specific nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-depended Fe^{3+} chelate reductase [1, 2]. (ii) Used by the Poaceae species involves the release of molecules known as phytosiderophores, chelators with high affinity for Fe(III) [3]. Many works were interested in comparing the relative effectiveness of some techniques like foliar sprays and Fe-EDDHA seed treatment used to reduce iron chlorosis [4, 5]. However, they are highly costing and did not improve iron nutrition plant [6, 7]. Thus, cultivar selection remains the most practical control measure for the iron deficiency chlorosis of plants. Our current study aimed to investigate the effect of induced iron deficiency on morphological and physiological responses of two apiaceous species (*Petroselinum crispum* Mill. and *Apium graveolens* L.).

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2. MATERIAL AND METHODS

2.1. Plant Material

After germination for one week in Petri dishes, *P. crispum* and *A. graveolens* seedlings were transferred to a half strength aerated hydroponic nutrient solution diluted 4 fold for 7 days. Then, similar sized seedlings were transferred into a continuously aerated half-strength Hoagland solution. The nutrient solution was composed of 2.5mM $Ca(NO_3)_2$, 3mM KNO_3, 1mM $MgSO_4$, 1mM KH_2PO_4, 20µM H_3BO_3, 2µM $MnSO_4$, 1µM $ZnSO_4$, 0.1µM $(NH_4)_6Mo_7O_{24}$ and 1µM $CuSO_4$. Two treatments were employed: 48.8µM Fe (Control, +Fe), pH=6 and 48.8µM Fe + 0.5 g/L $CaCO_3$ (Indirect iron deficiency, DI), pH=8.3. Iron solutions (Fe-EDTA) were prepared according to Jacobson's method [8]. All experiments were carried for one month, in a growth chamber with a light intensity of 5000 lux, at a temperature (day/night) of 24/18°C, 60% relative humidity and a photoperiod of 14/10h.

2.2. Leaf Chlorosis Parameters

Two methods were used to evaluate the chlorotic status of young leaves. The first one was the non-destructive index of Gildersleeve and Ocumpaugh [9] based on the visual chlorosis symptoms with values ranging from 0 (no apparent chlorosis) to 4 (severe chlorosis with necrosis). The second method measured chlorophyll and carotenoid contents according to Torrecilas *et al*. [10].

2.3. pH Measurements

The evolution of the nutrient solution pH was monitored during the final 10 days using a pH meter

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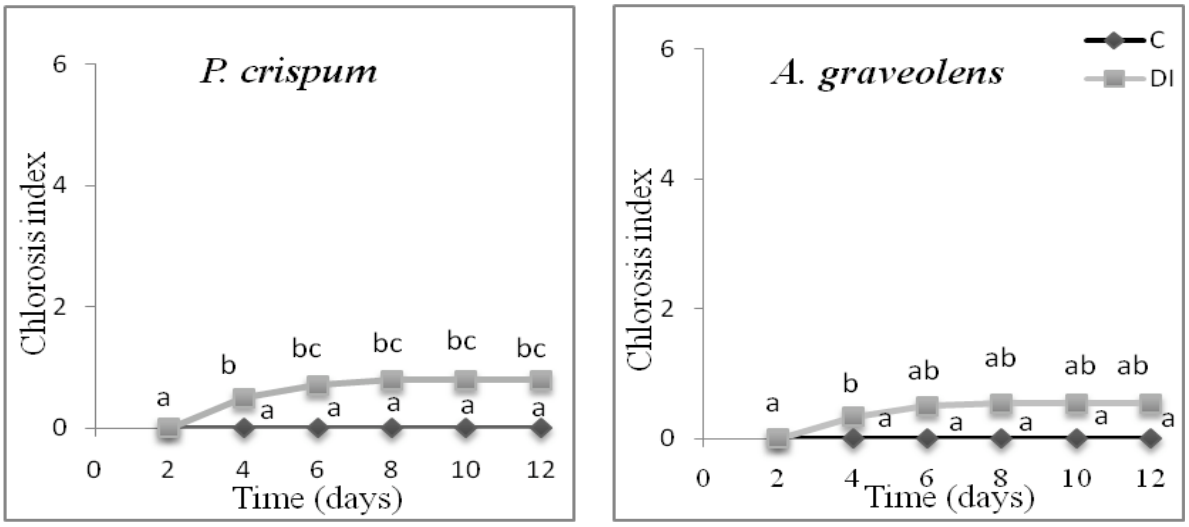

Figure 1: Chlorosis index according to Gildersleeve and Ocumpaugh scale in young leaves of *P. crispum* and *A. graveolens* cultivated with 48.8µM Fe or with 48.8µM Fe + $CaCO_3$ during 12 days. Different letters correspond to significantly different values at ($P<0.05$, n=24).

(Metrohm 84). The pH was initially fixed at 6 for the control and at 8.3 for ID medium.

2.4. Mineral Analysis and Plant Growth

After one month, plants were harvested and separated into shoots and roots. After rinsing roots with 1% (v/v) HCl, they were washed carefully with distilled water. Fresh weight of different plant organs was measured, then samples were dried at 70°C for 72 hrs and dry weights were determined. Mineral composition of each plant fraction was determined according to Zorrig *et al.* [11]. Samples were analyzed for micronutrient (Fe, Zn and Cu) by means of an Atomic Absorption Spectrophotometer (VARIAN 220 FS).

2.5. Statistical Analysis

The comparison of the data between species and treatments was performed using the SPSS 20.0 program. Means were compared using the Duncan's test at $P \leq 0.05$ when significant differences were found. Data shown are means of twenty four (chlorosis index), four (nutrient analysis) and eight (leaves number, shoot and root length, acidification capacity, plant dry weight and chlorophyll) replicates for each treatment.

3. RESULTS

3.1. Chlorosis Index and Chlorophyll Status

No chlorosis appearance was observed in young leaves of plants cultivated in nutrient solution containing bicarbonate, during the two first days of treatment (Figure **1**). The highest values of chlorosis score were recorded in *P. crispum* leaves. At the beginning of the treatment, this score increased slowly, and then was stabilized at about 0.79 and 0.54 respectively in deficient plants of *P. crispum* and *A. graveolens*.

As for the chlorosis index, a significant difference was noticed between the two species in terms of chlorophyll concentrations in young leaves. *A. graveolens* maintained a better chlorophyll status under iron deficiency conditions than *P. crispum*. Carotenoids content decreased by half in celery leaves, whereas it displayed no significant variation in *P. crispum* leaves (Figure **2**).

3.2. Root Acidification Capacity

Decreasing pH values were found in the nutrient solution of deficient plants of both species. pH values were significantly reduced in celery reaching 7.33 pH unit after two days of the treatment, while it does not exceed 7.73 in the case of parsley (Figure **3**).

3.3. Plant Growth

Leaves number per plant were slightly reduced under induced iron deficiency conditions for both species. Reduction percentage does not exceed 5.16%. No significant variation was registered in shoot length. However, root growth was significantly enhanced respectively, by 11.79% and 9.05% for parsley and celery (Table **1**). The whole plant dry matter deposition was markedly restricted by the bicarbonate induced Fe deficiency treatment; this

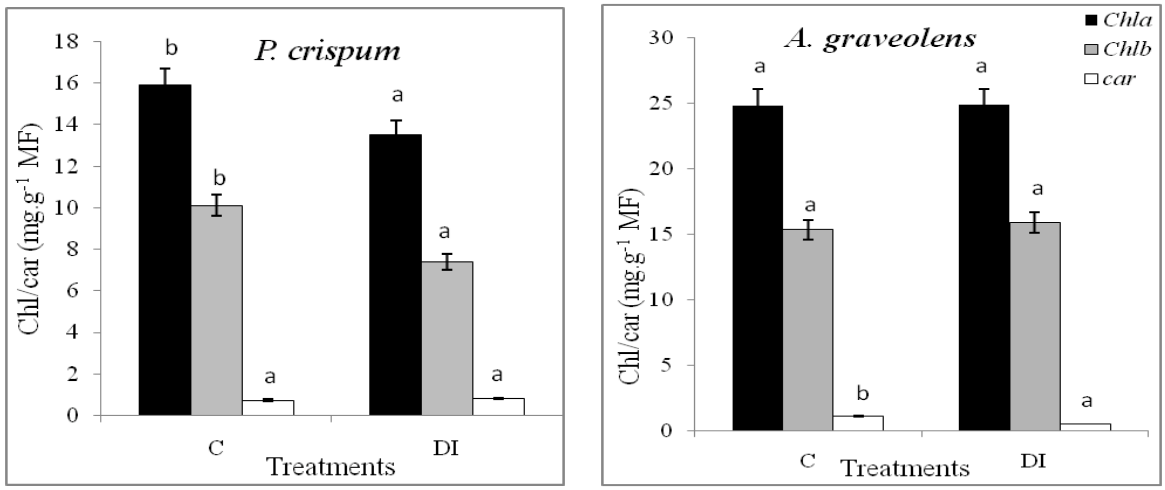

Figure 2: Chlorophyll and carotenoid content of the young leaves of *P. crispum* and *A. graveolens* grown during 30 days on a control nutrient solution (C), containing 48.8µM Fe, or under indirect iron deficiency (DI), containing 48.8µM Fe + $CaCO_3$. Values are means of 8 replicates ± standard deviation. Different letters correspond to significantly different values at P<0.05.

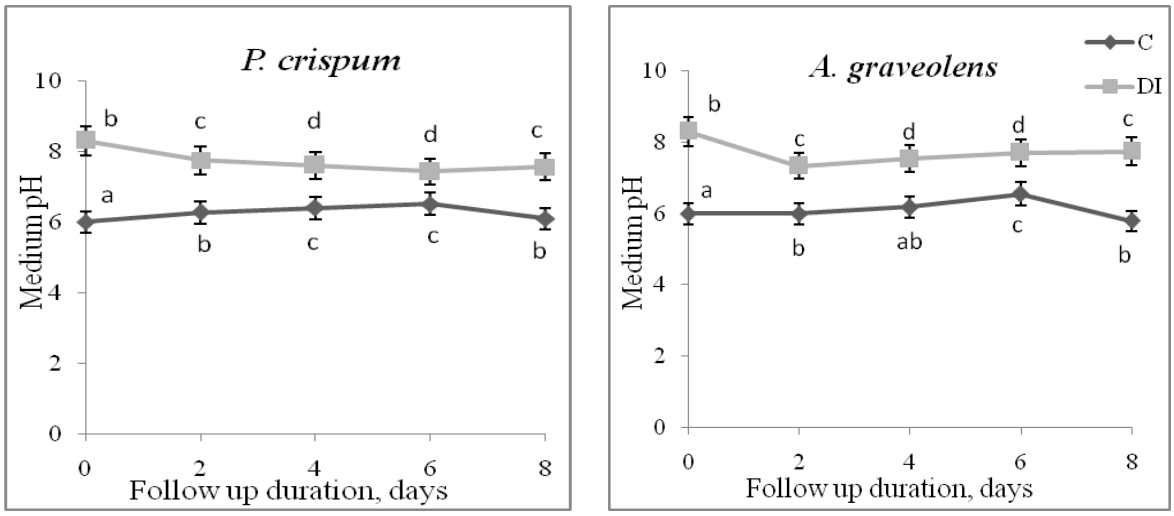

Figure 3: Changes in nutrient solution pH of control and deficient plant during the treatment period. Values are means of 8 replicates ± standard deviation. Different letters correspond to significantly different values at P<0.05.

restriction was more severe in parsley than in celery. A considerable decline in leaf dry weight was more pronounced in parsley (15%) than in celery (8%). Root biomass was reduced by 13.37% in parsley and 2.77% in celery in comparison to their respective controls. In addition, stem biomass was restricted by the same treatment (11.4% for parsley and 6.5% for celery) (Table **1**).

3.4. Nutrient Status

The effect of the treatment was more severe in roots than in shoots. Our results showed that iron content was strongly limited in all celery organs. An average reduction of 22.98% was registered in celery shoots (leaves and stems) and roots. However, this rate does not exceed 9% in parsley stems and roots (Table **2**). As shown in table **2**, no significant accumulation of Zn was registered in shoots for both species cultivated under deficient conditions. However, an increase of 19.76% was recorded in *P. crispum* roots. Copper accumulation was detected only in parsley roots and celery leaves cultivated under indirect iron deficiency, as compared to the control. In fact, the copper content was increased by 2.59 fold in parsley roots and by 2.7 fold in celery leaves, as compared to the control. The accumulation of this nutrient was organ and species dependent (Table **2**).

4. DISCUSSION

In this study, two apiaceous species grown in hydroponic culture were subjected to induced iron deficiency during one month. Significant differences

Table 1: Plant Growth in *P. Crispum* and *A. Graveolens* Grown during one Month on a Control Nutrient Solution Containing 48.8µM Fe (C: Control) or in the Presence of Fe and $CaCO_3$ (DI: Indirect Deficiency)

Species	*P. crispum*		*A. graveolens*	
Treatments	**C**	**DI**	**C**	**DI**
Leaves number	48.37 ± 9.16[b]	45.87±14.74[b]	42.25 ± 4.43[a]	41.12±6.31[a]
Shoot length(cm)	38.71 ± 3.18[a]	39.30±6.71[a]	55.55 ± 3.35[a]	52.55±3.92[a]
Root length (cm)	20.68 ± 2.34[a]	23.12±5.29[ab]	27.71 ± 6.48[ab]	30.22±8.16[ab]
Leaves DW (g)	3.49 ± 0.66[b]	2.95 ±0.52[a]	2.22 ± 0.55[b]	2.04 ±0.23[a]
Stem DW (g)	2.8 ± 0.58[b]	2.48 ±0.58[a]	2.59 ± 0.40[b]	2.42 ±0.28[b]
Root DW (g)	1.72 ± 0.58[b]	1.49 ±0.58[a]	1.08 ± 0.11[a]	1.05 ±0.15[a]

Different letters correspond to significantly different values at ($p<0.05$) according to Duncan test.

Table 2: Iron, Zinc and Copper Concentration (µg g^{-1}DW) in Shoots and Roots of *P. Crispum* and *A. Graveolens* Plants Grown for One Month under Iron-Sufficient (C: Control) or Iron-Deficient Medium (DI: Indirect Deficiency)

Species	*P. crispum*		*A. graveolens*	
Treatments	**C**	**DI**	**C**	**DI**
	Fe			
Leaves	6.99 ± 0.83[b]	8.23±1.2[b]	4.35 ± 0.43[b]	4.06±0.52[b]
Stems	4.08 ± 1.1[a]	4.02±4.62[a]	3.73 ± 0.91[b]	2.25±0.94[a]
Roots	11.63 ± 2.84[b]	9.64±1.45[b]	9.55 ± 3.02[b]	7.39±6.83[a]
	Zn			
Leaves	1.7 ± 0.19[a]	1.54±0.94[a]	1.12 ± 0.68[a]	1.08±0.70[a]
Stems	1.88 ± 1.36[a]	1.67±1.15[a]	1.22 ± 0.41[a]	0.772±0.14[b]
Roots	1.67 ± 0.20[a]	2±0.28[b]	1.19 ± 0.15[a]	1±0.26[a]
	Cu			
Leaves	0.655 ± 0.15[a]	0.55±0.41[a]	0.27 ± 0.17[a]	0.737±0.57[b]
Stems	1.06 ± 1.45[a]	0.23±0.06[b]	0.297 ± 0.05[a]	0.212±0.08[a]
Roots	1.49 ± 0.80[a]	3.86±0.80[b]	11.73 ± 22.26[b]	1.96±0.49[a]

Values are means of 4 ± S.D. Different letters correspond to significantly different values at ($p<0.05$) according to Duncan test.

between the two species in their capacity to acidify the rhizosphere, to maintain chlorophyll and iron content, also to develop root length were recorded. As shown above in our results, plant growth (leaf number, shoot length and biomass production) was significantly restricted in both species subjected to induced iron deficiency but with higher tolerance of celery. These results are in agreement with previous reports in chickpea [12], grapevine [13], pea [14], citrus [15] and medicago [16]. The lower biomass production observed under Fe deficiency is also partly ascribed to a decrease of chlorophyll concentration, as reflected by the yellowing of the youngest leaves of Fe deficient *P. crispum* and *A. graveolens* plants. Our results showed that chlorophyll content decreased significantly in *P. crispum* compared to *A. graveolens*. Our finding agrees with those obtained by [17, 18, 19]. Spiller and Terry [20] suggests that iron deficiency retards not only chlorophyll synthesis but also the synthesis of the complete light harvesting apparatus, including chloroplast membranes and the chlorophyll protein complexes, carotenoids, reaction center and electron carriers associated with them. As documented in several others species [14, 17], iron deficiency affected mineral nutrition in plants such as iron, zinc and copper content. The observed decline of iron content was more severe in celery organs than parsley. However, an accumulation of zinc and copper was observed in leaves and roots. The decrease of iron content, under limited conditions of this element, could be attributed to the bicarbonate effect [21]. Cohen *et al*. [22] reported that under iron deficiency, plants are able to uptake other micronutrients such as Zn and Cd.

As an adaptive trait used by plant to uptake nutrients from the soil, root length was increased under Fe deficient conditions. In our work, such result was recorded in both species which is in agreement with works on several plants species [23, 24]. Roots, the first organ in direct contact with the rhizosphere, are able to change the physicochemical properties of the rhizosphere *via* the release of H+ protons or the exudation of organic compounds. pH decrease was slightly more pronounced in celery than in parsley. Also, changes in pH is considered as the principal engine of nutrient uptake for plants [25] because it is responsible for the plasma membrane proton motive force and leads to the solubility of nutrients. Under limited conditions of iron in soils, in strategy I plants, the activation of plasma membrane proton pumps (H+-AT Pases) induced an establishment of an electrochemical gradient [26, 27] and lead to an increase of ferric Fe solubility [28]. Lowering the rhizosphere pH is considered as a good criterion of tolerance to iron deficiency.

CONCLUSION

The present work clarified some morphological and physiological responses of two apiaceous species to indirect iron deficiency. We could conclude that *A. graveolens* proved to be relatively more tolerant to iron deficiency than *P. crispum*. It revealed able to maintain plant growth and to acidify the culture medium. Future research should be directed to investigate biochemical and molecular responses.

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