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Iron Efficiency in Different Cucumber Cultivars: The Importance of Optimizing the Use of Foliar Iron

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Abstract. The ability of plant cultivars to deal with iron (Fe) deficiency (plant Fe efficiency capabilities) has a significant influence on crop yield and fruit quality. This study investigates Fe efficiency in four cucumber (*Cucumis sativus* L.) cultivars (Ashley, Anico, Trópico, and Serena) using a complementary approach. The ability to express the main Fe-stress root responses (rhizosphere acidification, Fe reduction, and specific morphological changes) and grow (dry matter production, nutrient acquisition, and efficiency of photosystem II) under Fe starvation were assessed. Results show that while the four cultivars were able to activate the main Fe-stress root responses, only 'Ashley' presented a significant capacity to grow and acquire nutrients under Fe deficiency. This ability to develop under Fe starvation was also reflected in the efficiency of photosystem II. Results suggest the existence of two different but probably inter-regulated mechanisms to deal with Fe deficiency in these plants. One would be related to the control of the expression of Fe-stress responses in the root and the other would be involved in optimizing the use of Fe present in the shoot, which is reflected in the plant's ability to develop under Fe deficiency. Among the cucumber cultivars studied, only 'Ashley' presented complete Fe efficiency, expressed in the development of Fe-stress root responses and in the ability to optimize Fe metabolic efficiency in the shoot. Likewise, this study evidences the need to consider Fe-stress root responses and the capacity to optimize the metabolic use of Fe in the shoot in studying plant Fe efficiency.

Cucumber is one of the most widespread horticultural crops in countries with a Mediterranean climate (Mediterranean basin, western North America, Chile, South Africa, and South Australia). About 90% of cucumber cultivation in the Mediterranean Basin takes place in greenhouses under conditions of intensive production, using alkaline soils with high calcium carbonate content [Ministry of Agriculture, Fisheries and Food (MAPA), 2004]. Iron deficiency (Fe chlorosis) is one of the most serious problems for horticultural crops, including cucumber, and fruit trees cultivated in alkaline and calcareous soils (Álvarez-Fernández et al., 2006; Lucena et al., 2007; Tagliavini and Rombolà, 2001). Two main strategies can be used to correct Fe deficiency in commercial crops. The most effective one is to provide readily available Fe for plants through the use of iron compounds capable of maintaining a sufficient level of soluble Fe in soil solution (Abadía et al., 2004; Lucena, 2006). Among these compounds, certain classes of Fe chelates have proven to be highly efficient (Lucena, 2006). However, the high cost of Fe chelates and similar compounds makes this strategy suitable only for high value crops, primarily fruit trees (Abadía et al., 2004).

Alternatively, a second strategy is based on growing plant cultivars resistant to Fe chlorosis. This strategy may be important in the case of horticultural and field crops (Hansen et al., 2006; Jolley et al., 2004).

Although the Fe-efficiency capabilities of certain cucumber cultivars have been characterized for research purposes, some authors highlighted the need for developing specific studies to better understand the real Fe efficiency of cucumber cultivars commonly used in Mediterranean climate countries (Cadahía, 1998). It is clear that such a study would be of great interest for seed producers and farmers.

In general, plant Fe efficiency has been related to the plant's capacity to develop specific physiological and morphological responses at the root level under Fe-deficient conditions (Briat, 2008; Römheld and Marschner, 1986; Schmidt, 2006). The so-called "Strategy I" plants (nongraminaceous monocots and dicots) activate physiological and biochemical responses that include the development of subapical swelling with abundant root hairs, transfer cells, the increase of Fe^{3+} enzymatic reduction at the root surface, the acidification of the rhizosphere, the increase in Fe^{2+} transporters, and the release of organic molecules with reducing capacity (Bienfait, 1988; Briat, 2008; Curie and Briat, 2003; Hell and Stephan, 2003; Jin et al., 2007; Römheld and Marschner, 1986; Schmidt, 2006). In recent years, major advances regarding the control at the molecular level of some of these responses have been made (Barton and Abadía, 2006; Briat, 2008; Curie and Briat, 2003). The genes encoding some of the enzymes and Fe transporters involved in root Fe uptake have also been characterized in certain plant species (Briat, 2008; Curie and Briat, 2003; Fox and Guerinot, 1998; Robinson et al., 1999; Vert et al., 2002; Waters et al., 2002). The main response of the Strategy II plants (graminaceous

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monocots) under Fe deficiency is characterized by the increase in the production and release to the rhizosphere of a specific family of organic molecules named phytosiderophores that have the capacity to form soluble Fe complexes, thus increasing Fe bioavailability (Briat, 2008; Kobayashi et al., 2006; Mori, 1999). In fact, some studies indicate that the concentration of Fe-phytosiderophore transporters in Strategy II plants also increases under Fe deficiency (Briat, 2008; Kobayashi et al., 2006).

However, a number of studies have shown that the capacity of certain plant cultivars to better grow under conditions of Fe deficiency was not clearly related to any of the Fe-deficiency root responses (De la Guardia and Alcántara, 2002). This fact suggested that other mechanisms could also be involved in the expression of Fe-efficiency capacities in these plants. These mechanisms may probably be related to the special ability of these plants to optimize the metabolic use of the Fe present in the shoot (Briat et al., 2007). This optimization of the Fe metabolic use in the shoot would be reflected in the shoot growth rate and in the amount of Fe extracted from the shoot under Fe-limiting conditions.

In this context, the aim of our work was to characterize the Fe-efficiency capacities of four commercial cucumber cultivars commonly used in the Mediterranean area (Serena, Trópico, Ashley, and Anico). We have considered the relationships of two complementary strategies. On the one hand, we have studied the capacity of these cucumber cultivars to develop the main Fe-stress root responses (reductase activity, rhizosphere acidification, and root morphological changes). On the other hand, we have evaluated the growth and nutrient extraction of the different cucumber cultivars under conditions of Fe deficiency (plant growth, nutrient extraction, leaf chlorophyll concentration, and efficiency of photosystem II).

Materials and Methods

Plant material and growth conditions

Four commercial cultivars of cucumber were used in the experiments: Serena, Anico, Trópico, and Ashley. The selection of the commercial cultivars was based on their utilization in intensive agriculture in Mediterranean climate countries (P. Monton, personal communication). The plants were cultivated in a growth chamber (irradiance: 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at a photoperiod of 15/9 h day/night, an average temperature of 23/21 °C day/night, and a relative humidity of 60% to 75%.

The nutrient solution used in the experiments was that described by Romera et al. (1999): 2 mM $Ca(NO_3)_2$, 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.5 mM KH_2PO_4, 50 μM KCl, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, and 0.35 μM Na_2MoO_4. The Fe source used in the treatments containing Fe was Fe-HBED (Fe-N, N' di-ortho hydroxibenzyl ethylene diamine diacetate).

Experimental design

The different plant seeds were germinated in perlite irrigated with a solution containing 1 mM calcium sulfate. During the 2 weeks following postgermination, the plants were irrigated with an aerated one-third–strength complete nutrient solution containing 1 μM Fe. The following week, the different treatments commenced: plants were separated into two groups corresponding to the two different treatments, one without Fe (–Fe) and the other with 13.3 μM Fe (+Fe). The plants were cultivated in perlite and were subirrigated with a one-third–strength complete nutrient solution. After 1 week, plants were transferred to vessels containing an aerated full-strength nutrient solution, and the concentration of Fe in the +Fe treatment was increased to the final concentration (40 μM). After 3, 5, 8, 10, 12, 16, 19, 23, and 26 d from the onset of the treatments, a specific number of plants from each treatment was separated to carry out the evaluation of some of the different parameters measured (12 plants in the case of the determinations corresponding to 3, 5, 8 and 10 d, and 6 plants for the other days of sampling). This experimental design was developed according to the results obtained in previous experiments (five independent experiments; data not shown) carried out to favor the gradual evolvement of leaf chlorosis and the plant root responses under Fe deficiency, as well as to obtain plants with an adequate size. The whole experiment was carried out twice and representative results are presented.

Parameters evaluated in the experiments

The effect of Fe deficiency on plant development was evaluated through the analysis of the following parameters.

Fe deficiency root responses. The plant capacity to acidify the rhizosphere was evaluated studying pH changes in the nutrient solution over time (Pinton et al., 1999; Romera and Alcántara, 2004).

The Fe(III)-chelate reductase activity in the root was measured by the method described in Pinton et al. (1999) using the bathophenanthrolinedisulfonate (BPDS) reagent. Roots of a single plant (1 g) were incubated in 5 mL of the nutrient solution containing 0.387 mM Fe (III)-EDTA and 0.286 mM BPDS (pH 5.5) in darkness at 25 °C. After 30 min, the absorbance of the solution was measured at 535 nm and the quantity of Fe(III) reduced was calculated by the concentration of the Fe(II)-BPDS complex formed, using an extinction coefficient of 22.1×10^{-3} $M^{-1} \cdot cm^{-1}$. We observed in previous experiments a good reproducibility of the results obtained using this method, as well as a good correlation between these results and those obtained using the whole root of the plant (data not shown).

Subapical swelling and the development of root hairs were measured using optical microscopy (×25, model SZX 12; Olympus, Tokyo) and image analysis program Leica DC Viewer – Qwin, (Leica, Cambridge, UK). To evaluate the evolution of these root morphological changes over time, we focused the study on the apical root area (30% of the root surface considered from the bottom of the root), examining principally new lateral, secondary roots. The results were quantitatively characterized as follows: 25% of the surface of the lateral secondary root analyzed covered with root hairs (+), 50% (+ +), 75% (+ + +), and 100% (+ + + +). The absence of development of root hairs is expressed as (–).

Fe deficiency leaf responses. The leaf concentration of Chl was determined using the portable instrument SPAD-502 m (Minolta, Osaka, Japan). Previously, a calibration curve was established measuring the Chl concentration corresponding to a selected group of SPAD readings. Chl leaf concentration was determined using the method described in Séstak et al. (1971).

Chl fluorescence measurements. The effect of Fe deficiency on plant photosynthetic efficiency was evaluated through the determination of the photosystem II (PSII) efficiency in dark-adapted (F_v/F_m) and illuminated leaves ($\phi_{PSII} = F'_q/F'_m$), the intrinsic PSII efficiency ($\phi_{exc} = F'_v/F'_m$), the

photochemical [qP = $(F'_m - F_s)/F'_v$)] and nonphotochemical [NPQ = $(F_m/F'_m) - 1$] quenching, and the fraction of light absorbed by PSII that is dissipated in the antenna [D (identical to $1 - \phi_{exc}$)]. Chl fluorescence parameters (F_0 and F'_0, basal fluorescence from dark-adapted and illuminated leaves; F_m and F'_m, maximal fluorescence from dark-adapted and illuminated leaves; F_v and F'_v, variable fluorescence from dark-adapted and illuminated leaves; F', fluorescence emission from leaf adapted to actinic light; F'_q, and difference in fluorescence between F'_m and F') were measured with a portable fluorimeter (FMS 2; Hansatech, Kings Lynn, UK) and following the method described by Schreiber et al. (1986).

Plant growth. After 3, 5, 8, 10, 12, 16, 19, 23, and 26 d, a minimum of four plants belonging to the different treatments were harvested. First they were weighed, then one part of the shoot and roots of the plants was oven dried (40 °C) in paper bags for dry weight determination and further mineral analysis.

Plant nutrient uptake. The mineral nutrient concentration was analyzed in dried samples of roots and shoots using emission optical spectroscopy with inductively coupled plasma (EOS-ICP), after microwave acid sample digestion.

Statistical analysis

The results are presented as the mean ± SE. All experiments were carried out in triplicate, and data were analyzed statistically by analysis of variance, followed by the Fisher's test when necessary, with the level of significance set at $P < 0.05$. The significant differences between treatments (–Fe vs. +Fe) or within a specific treatment were described by an asterisk.

Results

In the Tables and Figures, the results are presented after 12 d of the onset of Fe deficiency, when the differences between Fe-sufficient and Fe-deficient plants were significant. In the case of rhizosphere acidification, pH measurements were finished 23 d after the onset of Fe deficiency, coinciding with the renewal of the nutrient solution.

Time course experiments of root responses to Fe deficiency. As shown in Fig. 1, the different cucumber cultivars presented a clear acidification of the nutrient solution after 16 d for 'Serena', 'Anico', and 'Trópico', and after 19 d in the case of 'Ashley'. The intensity of this response was similar in the case of 'Serena', 'Anico', and 'Trópico', and was slightly lower in the case of 'Ashley'.

Likewise, 'Serena', 'Anico', and (less markedly) 'Trópico' presented an increase in the Fe(III)-chelate reductase activity after 12 d from the onset of the treatments. However, 'Ashley' presented a significant increase in reductase activity after 19 d (Fig. 2). The intensity of this response varied according to the plant cultivar. 'Anico' plants presented more activity over time when compared with the other cultivars. 'Ashley' presented a similar intensity to that of 'Anico' but for a shorter time period. 'Trópico' plants showed a significant but rather irregular reductase activity over time. 'Serena' plants presented a significant increase in reductase activity but with a lower maximum intensity than that of the other cultivars.

As for the development of morphological changes in the root under Fe deficiency—subapical swelling and hair proliferation in lateral secondary roots—the results also varied according to the different plant cultivars (Table 1). Thus, 'Ashley' and 'Anico' presented differences between +Fe and –Fe plants after

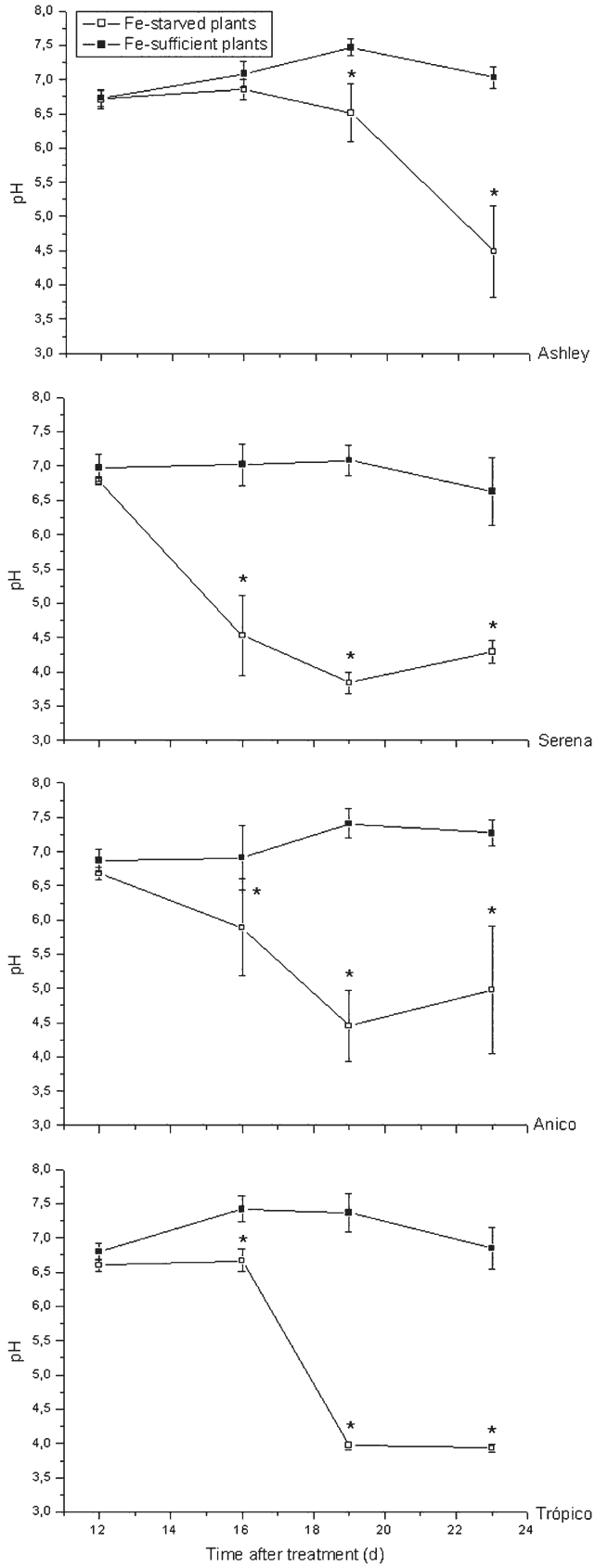

Fig. 1. pH variation (mean ± SE, n = 6) in the nutrient solution in Fe-starved (-□-) and Fe-sufficient (-■-) plants of four cucumber cultivars (Ashley, Serena, Anico, and Trópico). An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments.

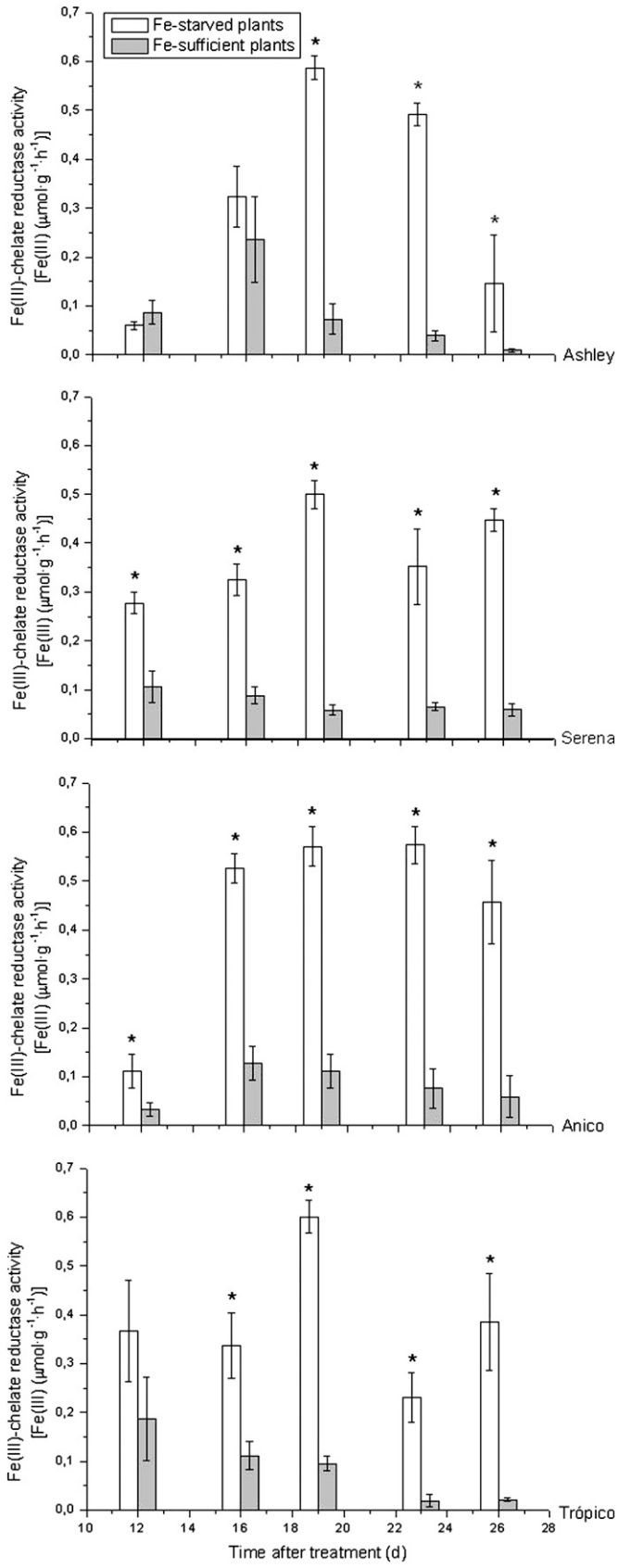

Fig. 2. Fe(III)-chelate reductase activity in roots of Fe-starved (-□-) and Fe-sufficient (-■-) plants of four cucumber cultivars (Ashley, Serena, Anico, and Trópico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

10 to 12 d from the onset of the treatments. Those differences became particularly clear after day 16. In 'Serena' plants, clear differences between +Fe and –Fe treatment were not noted. However, in this case, a certain hair development in the middle section of secondary roots and certain subapical swelling in lateral secondary roots were observed in Fe-starved and Fe-sufficient plants (Table 1). In 'Trópico' plants, slight differences were observed after 19 d.

Effects of Fe deficiency on Chl concentration in leaf. As presented in Fig. 3, all of the cucumber cultivars in this study evidenced a clear reduction in the concentration of Chl in leaves after 16 d from the onset of the experiments in Fe-starved plants. There were no clear differences among the different cultivars regarding Chl concentration in leaf.

Effects of Fe starvation on PSII operating efficiency. As far as PSII efficiency is concerned, the results showed that whereas only slight reductions in PSII efficiency in leaves adapted to darkness (PSII maximum quantum efficiency) were noted in Fe-starved plants (data not shown), the values of the actual PSII efficiency measured in illuminated leaves (ϕPSII), the photochemical quenching (qP), and the intrinsic PSII efficiency (ϕexc), were significantly reduced under conditions of Fe deficiency (Fig. 4). This reduction was different depending on the plant cultivar. In the case of Fe-starved 'Serena' and 'Trópico' plants, the decrease in qP was observed 12 d after the onset of Fe deficiency, while this reduction was confirmed after 16 d in 'Ashley' and 'Anico'.

Thus, Fe-starved 'Serena' plants presented a reduction in ϕPSII, associated with a reduction in qP (Fig. 4) and an increase in D (data not shown), 12 d from the onset of Fe deficiency. After 23 and 26 d, a clear decrease in ϕPSII was registered. This was linked to a decrease in qP (Fig. 4), a slight increase in D, and a significant increase in NPQ (data not shown). ϕexc values experienced a slight but significant reduction respect to the Fe-sufficient plants, except for the plants harvested after 19 d from the onset of the treatment (Fig. 4).

In the case of Fe-starved 'Trópico' plants, a clear decrease in ϕPSII was observed 16 d after the onset of Fe deficiency (data not shown). This result was accompanied with a decrease in qP and ϕexc values (Fig. 4) and an increase in D and NPQ (data not shown). These results were maintained after 19 d. ϕPSII values recovered after 23 d, a fact that was also reflected in an increase in qP and ϕexc values. However, after 26 d, a slight reduction in ϕPSII that was related to a decrease in qP but not in ϕexc was also observed (Fig. 4). This time, these results were also coupled with a slight increase in D but not in NPQ (data not shown).

In the case of Fe-starved 'Ashley' plants, a significant decrease in ϕPSII, qP, and ϕexc values was observed after 16 d and, more importantly, after 19 d from the onset of Fe deficiency (Fig. 4). These results were coupled with significant increases in D and NPQ values (data not shown). After 23 d, a clear recovery in ϕPSII was noted, although this parameter and also qP values were still significantly lower in Fe-deficient plants (Fig. 4). The recovery of ϕPSII, qP, and ϕexc values was complete after 26 d and the values were maintained after 30 d from the onset of Fe deficiency (data not shown).

Fe-starved 'Anico' plants presented a clearly marked decrease in ϕPSII 16 d after the onset of Fe deficiency (data not shown). This was accompanied with significant decreases in qP and ϕexc values (Fig. 4) and a clear increase in D and NPQ (data not shown). This behavior was maintained after 19 and

Table 1. Relative intensity of root morphological changes in Fe-starved (0 μM) and Fe-sufficient (40 μM) plants of four cucumber cultivars.

Cultivar	Fe treatment	Relative intensity of root morphological changes (– to ++++ scale)[z] 12 d	16 d	19 d	23 d	26 d
Ashley	0 μM	++	+++	++++	+++	++
	40 μM	+	—	—	—	—
Serena	0 μM	++	+++	+++	++	—
	40 μM	++	++	++	++	++
Anico	0 μM	++	+++	++++	++++	++++
	40 μM	—	—	—	—	—
Trópico	0 μM	++	—	+++	—	++
	40 μM	++	++	—	—	—

[z]–, +, ++, +++, or ++++ indicate 0%, 25%, 50%, 75%, or 100% of the surface of the adventitious root covered with root hairs, respectively.

23 d. A certain recovery in ϕPSII occurred after 26 d, which was reflected in qP and ϕexc increase (Fig. 4). However, this recovery was transient and it was not maintained 30 d after the onset of Fe deficiency (data not shown).

Effects of Fe deficiency on plant growth. As far as plant growth is concerned, all of the cucumber cultivars experienced a significant reduction in growth of the root and the aerial part under conditions of Fe deficiency (Fig. 5). However, 'Ashley' plants growing under Fe deficiency presented a progressive and significant growth of roots and shoot (Fig. 5). 'Serena' and 'Trópico' also presented a slight and progressive increase in the growth of the aerial part in plants under Fe deficiency, although this increase was clearly lower than that of 'Ashley' plants and was globally nonsignificant ($P < 0.05$) (Fig. 5). Finally, 'Anico' plants did not present any growth at all in the roots or the aerial part of the plant under Fe deficiency (Fig. 5).

Plant nutrient uptake as a function of nutrient extraction from the shoot. To facilitate the analysis of the data related to plant nutrient acquisition, the results were presented as a function of the nutrient extraction from the shoot. This type of data presentation was chosen because, in many cases, there were no clear differences in nutrient concentration between the root and the shoot in Fe-sufficient and Fe-deficient plants due to a concentration effect associated with the significant decrease in dry matter production for Fe-deficient plants.

Concerning P, K, Ca, and Mg, Fe-deficient 'Ashley' plants developed a reduced extraction of these nutrients only after 23 d from the onset of the treatments. However, this decrease was not observed in the following harvest (26 d) except in the case of P (Fig. 6). In the case of Fe-starved 'Anico' plants, a clear and maintained decrease in the extraction of the studied nutrients was observed 23 d after the onset of Fe deficiency (Fig. 6).

In the case of Fe and the other main micronutrients studied (Mn, Zn, B, and Cu), Fe-starved 'Ashley' plants presented a significant decrease in Fe extraction from the shoot 16 d after the onset of treatments (Fig. 7). However, a significant increase in Fe assimilation was observed after 19 d in these plants.

Fe-starved 'Anico' plants presented no significant increase in Fe assimilation (Fig. 7). Regarding micronutrient shoot extraction in Fe-starved 'Ashley' plants, extraction of Zn and Mn increased after 16 d from the onset of the treatments, and this increase remained significant for the rest of the experiment (Fig. 7). The same was observed in the case of Cu, but only at 16 and 26 d from the onset of the treatments (Fig. 7). In the case of Fe-starved 'Anico' plants, a similar pattern of shoot extraction was observed at 12, 16, 19, and 23 d from the onset of the treatments for Mn and Cu (Fig. 7).

Discussion

Our experiments suggest that all the cucumber cultivars in this study were able to activate the main Fe-stress root responses under conditions of Fe-starvation (Figs. 1 and 2, Table 1). However, time course results and the intensity of root responses were different depending on the plant cultivar, as will be discussed later. Consequently, the four cucumber cultivars are Fe efficient, in concordance with the most widely accepted theory that relates Fe efficiency to the expression of Fe-stress root responses. However, regarding plant growth and nutrient acquisition under Fe-deficient conditions, our results show that among the different cucumber cultivars studied, 'Serena' and 'Trópico' were able to grow mildly under Fe-deficient conditions, while 'Anico' showed a clear inefficiency and 'Ashley' presented the highest efficiency (Fig. 5). Likewise, 'Ashley' was able to maintain root nutrient uptake activity and nutrient translocation under conditions of Fe starvation (Figs. 6 and 7). In fact, there were no clear differences between Fe-sufficient and -deficient 'Ashley' plants in relation to shoot extraction of P, Ca, and Mg, except for the case of the plants harvested 23 d after the onset of the treatments. An increase in shoot extraction of Mn, Zn, and Cu in Fe-starved 'Ashley' plants was observed that could be associated with a reduction in the competition between Fe and those micronutrients in the root uptake process (Fig. 7) (Marschner, 1995). Moreover, certain studies showed that some of those micronutrients could also be a substrate for the root chelate-reductase (Lucena et al., 2003). In the case of Fe-starved 'Anico' plants, the clear incapacity to grow under Fe starvation was also reflected in a reduction in plant nutrient uptake activity (Fig. 5). This different efficiency to grow under Fe deficiency between 'Ashley' and 'Anico' plants is also reflected in the significant and sustained recovery in ϕ_{PSII} values observed in Fe-starved 'Ashley' plants, which was accompanied with a correspondent recovery in qP and ϕ_{exc} (Fig. 4). In 'Anico' plants, only a transient recovery was observed (Fig. 4).

However, there were no clear differences in the expression of the Fe-stress root responses between 'Ashley' and 'Anico' plants. In fact, 'Ashley' plants expressed root reductase activity and rhizosphere acidification after 'Anico', 'Trópico', and 'Serena' plants (Figs. 1 and 2). Likewise, 'Anico' plants presented the highest (and time sustained) chelate-reductase activity and rhizosphere acidification (Figs. 1 and 2). Thus, in our case, the time course and intensity of Fe-stress root responses seemed to be inversely related to the efficiency of the plant to grow under Fe starvation. This result suggests that the expression of Fe-stress root responses might be subordinated to the capacity of the plant to integrate the Fe present in the leaves in different metabolic and physiological processes (Briat et al., 2007). There were no significant differences in the

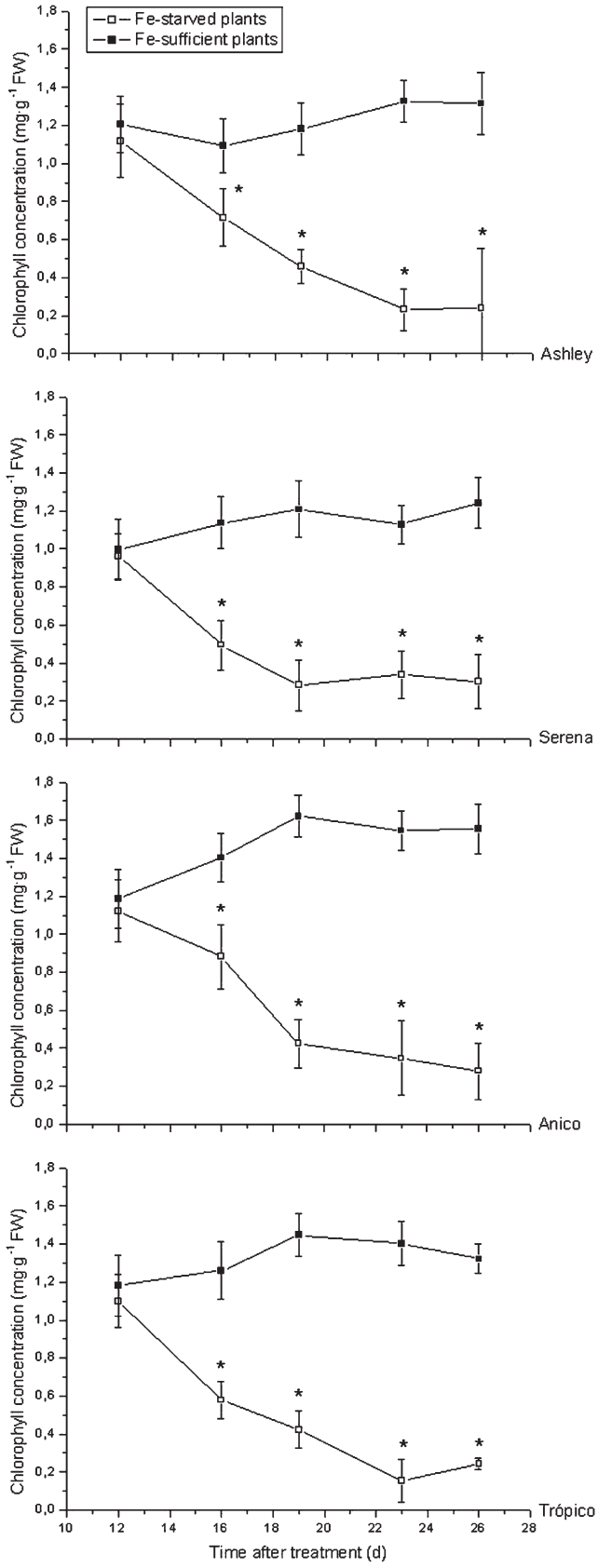

Fig. 3. Chlorophyll concentration variation in Fe-starved (-□-) and Fe-sufficient (-■-) plants of four cucumber cultivars (Ashley, Serena, Anico, and Trópico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

shoot concentration of Fe associated with 'Anico', 'Trópico', 'Serena', and 'Ashley' plants at the onset of Fe starvation treatment (data not shown), although their capacity to grow under normal Fe availability was clearly different (Fig. 5). In the case of 'Ashley' plants, this capacity to grow under Fe deficiency was coupled with an increase in the Fe shoot extraction (Fig. 7), which suggests a better ability to use the Fe already present within the plant (Fe mobilization in the root and further translocation to the shoot).

In summary, these results show that there was not a direct link between the Fe efficiency of the plants, according to their capacity to develop under Fe starvation, and the inferred Fe efficiency from their capacity to express the main Fe-stress root responses in the cucumber cultivars in this study.

Other authors have also described this phenomenon. Thus, although a number of studies described a good relationship between the root chelate-reductase activity and the resistance to Fe deficiency in the field for soybean (*Glycine max* L.) (Jolley et al., 1992), dry bean (*Phaseolus vulgaris* L.) genotypes (Ellsworth et al., 1997, 1998), peach (*Prunus persica* L.) genotypes and peach rootstocks (De la Guardia et al., 1995; Romera et al., 1991), tomato (*Solanum lycopersicum* L.) genotypes (Dasgan et al., 2002), and citrus (*Citrus* L.) genotypes (Manthey et al., 1993); other studies did not find clear relationships between some of the Fe-stress root responses (Fe(III) reduction or rhizosphere acidification) and the efficiency to grow under Fe-deficient conditions for quince (*Cydonia oblonga* Mill.), pear (*Pyrus communis* L.), and olive (*Olea europaea* L.) (De la Guardia and Alcántara, 2002). Indeed, the cultivars of some olive trees presented clear Fe-efficiency capabilities according to their capacity to grow under Fe starvation and a very low root chelate-reductase activity under Fe deficiency (De la Guardia and Alcántara, 2002).

Our results suggest the existence of two different levels of Fe efficiency in these cucumber cultivars. The first level of Fe efficiency would be related to the ability of the plant to activate the main Fe-stress root responses under Fe deficiency. This response would be focused to obtain Fe from the rhizosphere and may be controlled by the amount of Fe within the root (Bienfait et al., 1987; Briat, 2008; Schmidt, 2006). Then, a second level of Fe efficiency would be reflected in the optimization of the use of those Fe molecules already present in the plant. This ability would be attested by the capacity of the plant to grow and maintain physiological activity under Fe deficiency. This pattern of response is compatible with those models proposing the existence of an interrelated system involving a local signal in the root and a systemic signal related to the Fe status in the shoot for the control of Fe-stress root responses (Briat, 2008; Vert et al., 2003).

Our results also indicate that Fe status in the shoot is mainly related to the capacity of the plant to integrate foliar Fe in metabolic processes rather than to the total Fe concentration in leaf because the four cucumber cultivars had similar Fe levels in roots and shoots at the onset of Fe starvation treatments. This conclusion is also in line with the results of a number of studies describing the development of leaf chlorosis in the presence of normal concentration of Fe in leaf (chlorosis paradox) although in many cases this fact can also be caused by a concentration effect due to a minor leaf growth (Römheld, 2000).

Regarding the nature of the signals involved in this regulatory process, a number of studies suggest that some plant

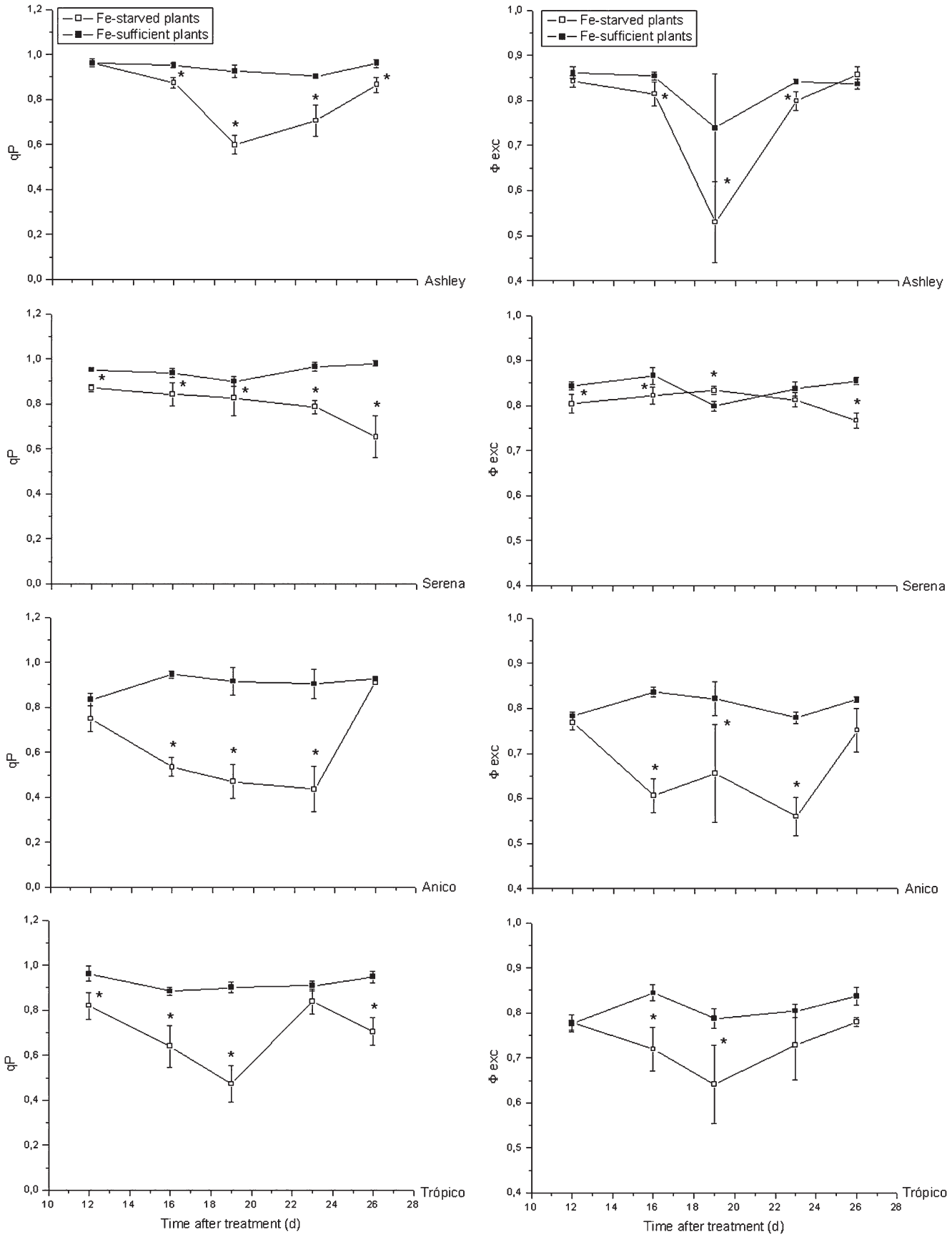

Fig. 4. Photochemical quenching (qP), and the intrinsic PSII efficiency (ϕexc), for Fe-starved (-□-) and Fe-sufficient (-■-) plants of four cucumber cultivars (Ashley, Serena, Anico, and Trópico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

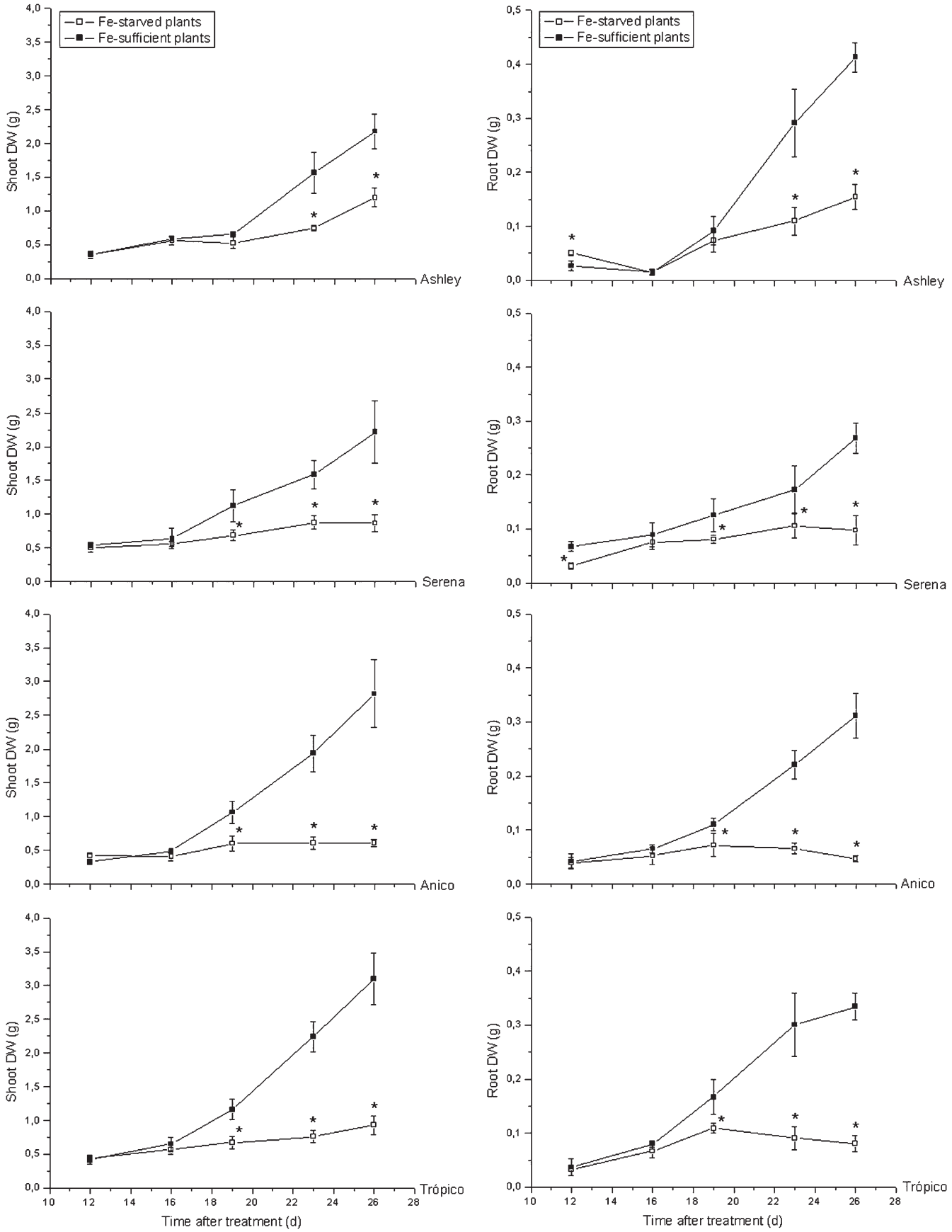

Fig. 5. Shoot and root growth in Fe-starved (-□-) and Fe-sufficient (-■-) plants of four cucumber cultivars (Ashley, Serena, Anico, and Trópico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

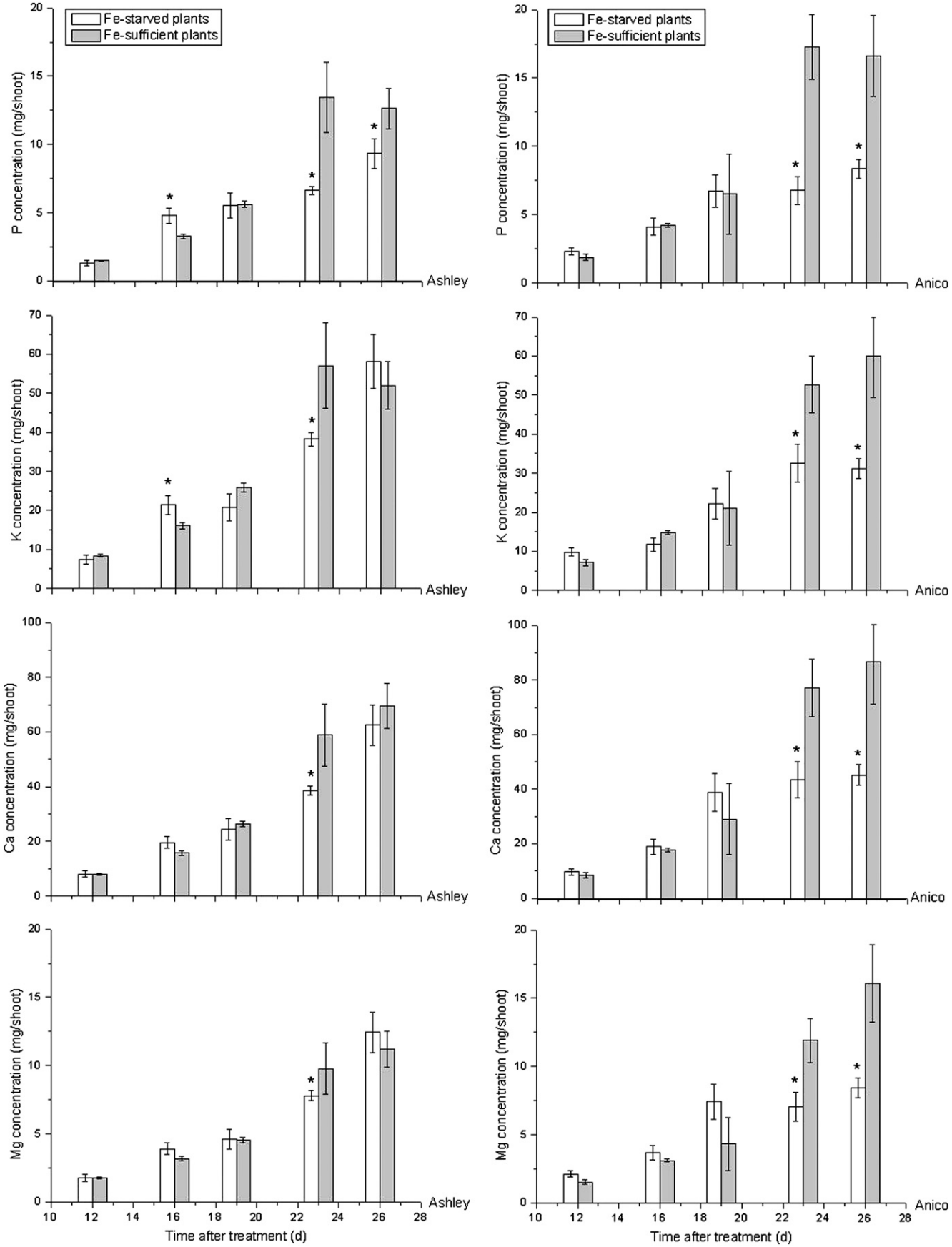

Fig. 6. Macronutrient concentration in shoot of Fe-starved (-□-) and Fe-sufficient (-■-) plants of two different cucumber cultivars (Ashley, Anico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

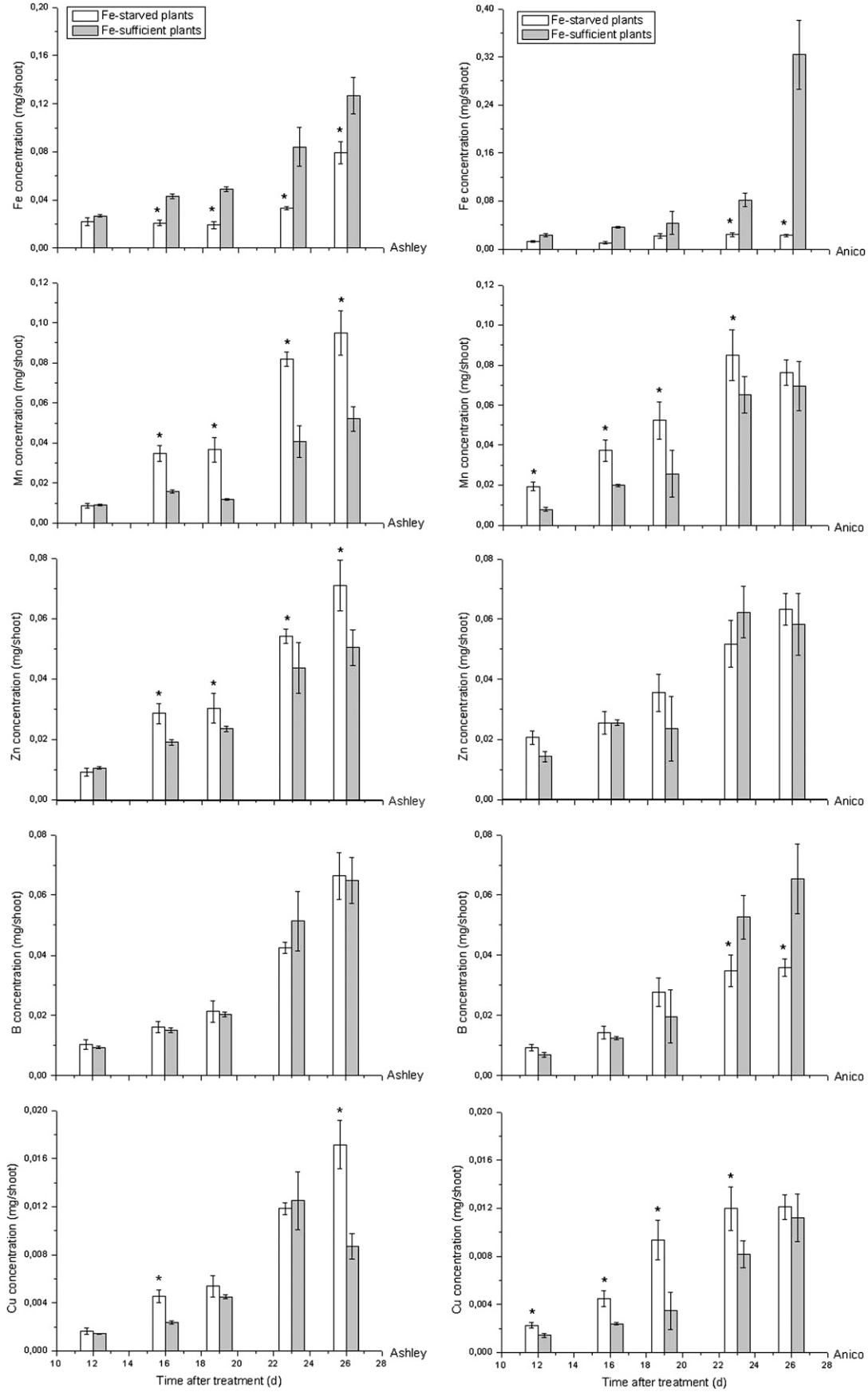

Fig. 7. Micronutrient concentration in shoot of Fe-starved (-□-) and Fe-sufficient (-■-) plants of two different cucumber cultivars (Ashley, Anico). (Mean ± SE, n = 6). (An asterisk indicates the presence of significant differences for $P < 0.05$ between +Fe and –Fe treatments).

regulators such as ethylene, nitric oxide, and auxin (Romera et al., 2006; Rubio et al., 2009) could affect the regulation of the expression of Fe stress plant responses. However, the actual role of those plant regulators remains unclear (Romera et al., 2006; Rubio et al., 2009). More studies are required to clarify the role of those hormones in the regulation of the plant responses when expressed under conditions of Fe deficiency.

In conclusion, our results show that despite all of the cucumber cultivars in this study showing the ability to activate Fe-stress root responses under Fe deficiency, only 'Ashley' presented a significant capacity to optimize the metabolic use of foliar Fe, which is reflected in its capacity to develop under Fe starvation. Likewise, our results also show that it would be necessary to consider the expression of Fe stress root responses and the plant's ability to develop under Fe starvation for a better evaluation of Fe use efficiency in plants.

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