



## Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution

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### Abstract

We studied the effects of Fe deficiency on physiological parameters of citrus rootstocks grown in nutrient solution. Three 4-week old rootstocks ('Troyer' citrange – *Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf., *Citrus taiwanica* Tan. and Shim., and 'Swingle' citrumelo – *Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.) were grown in nutrient solutions with 0, 5, 10, 15 and 20  $\mu\text{mol Fe dm}^{-3}$ . Calcium carbonate ( $1 \text{ g dm}^{-3}$ ) was added to all solutions to mimic the natural conditions in calcareous soils. For each rootstock, shoot length, number of leaves, and root and shoot dry weights were measured at the end of experiment. Chlorophyll concentration was estimated using a portable SPAD-502 meter calibrated for each rootstock. The amount of nutrients (P, K, Mg, Ca, Fe, Zn, Mn, and Cu) was determined in shoots. Chlorophyll fluorescence parameters ( $F_0$ : basal fluorescence;  $F_m$ : maximum fluorescence;  $F_v = F_m - F_0$ : variable fluorescence) were measured with a portable fluorimeter. 'Troyer' citrange rootstock was the most tolerant to Fe deficiency. These plants grew more and accumulated more chlorophyll and nutrients than the others in the presence of low levels of Fe ( $10 \mu\text{mol Fe dm}^{-3}$ ). 'Swingle' citrumelo plants needed  $20 \mu\text{mol Fe dm}^{-3}$  in the

*Abbreviations:* Chl, leaf chlorophyll; NS, not significant; P, level of significance;  $R^2$ , correlation coefficient

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nutrient solution to secure adequate growth. ‘Taiwanica’ orange rootstock had an intermediate behaviour, but could be distinguished from ‘Troyer’ citrange based on fluorescence parameters, since there was a variation in the basal fluorescence in the former, whereas in ‘Troyer’ citrange the basal fluorescence was not affected by the supply of Fe.

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## 1. Introduction

It is estimated that from 20 to 50% of fruit trees grown in the Mediterranean basin suffer from Fe deficiency (Jaegger et al., 2000). The most prevalent cause of Fe deficiency in this region is the presence of high levels of bicarbonate ions in the calcareous soils. These soils often have more than 20% of calcium and magnesium carbonates and are strongly buffered, with a pH between 7.5 and 8.5. The high level of bicarbonate ions in the soil affects metabolic processes in roots and leaves, decreasing soil and plant Fe availability (Mengel, 1995), leading to the condition known as lime-induced iron chlorosis. The most evident effect of Fe chlorosis is a decrease in photosynthetic pigments, resulting in a relative enrichment of carotenoids over chlorophylls (Chl), leading to the yellow colour characteristic of chlorotic leaves (Abadía and Abadía, 1993; Morales et al., 1998). When Fe is in short supply, Fe-efficient genotypes develop controlled responses, which include physiological, biochemical and morphological changes (Schmidt, 1999).

In fruit trees, lime-induced Fe deficiency results in considerable loss of yield (Pestana et al., 2003), delayed fruit ripening, and impaired fruit quality, as reported in peach (Sanz et al., 1997) and orange (Pestana et al., 2001a). The success of citrus production depends on the availability of suitable rootstocks. Their tolerance to lime-induced Fe chlorosis varies (Hamzé et al., 1986; Wutscher, 1979), with trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) being very susceptible and sour orange (*C. aurantium* L.), various mandarins (*C. reticulata* Blanco, *C. nobilis*), limes (*C. limonia* Osb.), and lemons (*C. jambhiri* Tan.) being more tolerant than trifoliolate orange. However, differential tolerance to other factors such as citrus tristeza virus limits the choice of rootstocks that can be used. Genetic improvement of rootstocks to enhance chlorosis-resistance would be the best solution to Fe chlorosis, but this is a long-term approach. Meanwhile, screening techniques to identify tolerant genotypes need further development, and for that additional information on morphological, physiological and molecular mechanisms involved in the different genetic responses to Fe chlorosis is required.

The screening of cultivars to iron deficiency in hydroponics systems with bicarbonate has been used for herbaceous (Alhendawi et al., 1997; Campbell and Nishio, 2000; Lucena, 2000; Pissaloux et al., 1995) and woody species (Alcántara et al., 2000; Cinelli et al., 1995; Nikolic et al., 2000; Romera et al., 1991a, 1991b; Shi et al., 1993; Sudahono et al., 1994).

The aim of this study was to evaluate the effects of Fe deficiency on three citrus rootstocks (‘Troyer’ citrange, ‘Taiwanica’ orange and ‘Swingle’ citrumelo) using different growth parameters and physiological characteristics.

## 2. Materials and methods

### 2.1. Plant material and growth

Three citrus rootstocks were studied: ‘Taiwanica’ orange (*Citrus taiwanica* Tan. and Shim.), ‘Troyer’ citrange (*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.) and ‘Swingle’ citrumelo (*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.). Seeds were obtained from Willits & Newcomb (Arvin, CA, USA), and were sterilised by immersion in a 15% sodium hypochlorite solution for 15 min and rinsed three times with running water. Seeds were germinated in the dark at 22 °C in plastic trays with sterilised moist vermiculite. After germination the seedlings were grown in moist vermiculite during 4 weeks in a controlled environment with day/night temperatures of 21/22 °C, a relative humidity of 80% and a 12 h photoperiod. The minimum photon flux density at plant level was 113  $\mu\text{mol}$  quanta photosynthetic active radiation (PAR)  $\text{m}^{-2} \text{s}^{-1}$ , provided by a combination of fluorescent and incandescent lamps. At the end of this period, groups of uniform seedlings (with three to four leaves and a height of approximately 7 cm) were selected for each rootstock, and placed in polystyrene boxes containing 20  $\text{dm}^{-3}$  of a nutrient solution with the following composition (in  $\text{mmol dm}^{-3}$ ): 5.0  $\text{Ca}(\text{NO}_3)_2$ , 1.4  $\text{KNO}_3$ , 0.6  $\text{K}_2\text{SO}_4$ , 1.0  $\text{MgSO}_4$ , 0.9  $\text{NaCl}$ , 0.6  $(\text{NH}_4)_2\text{HPO}_4$ , 3.0  $(\text{NH}_4)_2\text{SO}_4$ , 0.2  $\text{MgCl}_2$ , and (in  $\mu\text{mol dm}^{-3}$ ) 41.8  $\text{H}_3\text{BO}_3$ , 3.8  $\text{ZnSO}_4$ , 3.9  $\text{CuSO}_4$ , 6.9  $\text{MnSO}_4$  and 1.0  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  (Carpena, 1983). Iron was added to the solution as Fe(III) Na-EDTA at four different concentrations: 0, 5, 10, 15 and 20  $\mu\text{mol Fe dm}^{-3}$ . Calcium carbonate (1 g  $\text{CaCO}_3 \text{ dm}^{-3}$ ) was added to mimic the natural conditions in a calcareous soil. The nutrient solutions were constantly aerated. Due to the small size of the seedlings at the beginning of the experiment, half-strength solutions (with exception of Fe and  $\text{CaCO}_3$ ) were used over the first 25 days. The solutions were then replaced with full-strength solutions. The electrical conductivity and the pH of solution were monitored twice a week. After the addition of the  $\text{CaCO}_3$  the pH of the solutions was  $7.2 \pm 0.1$ . Nutrient solutions were replaced twice a month when the electrical conductivity dropped to 1.0  $\text{dS m}^{-1}$  (half-strength) or 1.7  $\text{dS m}^{-1}$  (full-strength nutrient solution). Plants were grown in a glasshouse for 9 weeks under natural photoperiod conditions and temperature  $\leq 25$  °C. For each treatment two containers were used, with four plants of each rootstock per container. So, in the total there were 10 containers and eight plant replications per treatment.

### 2.2. Leaf chlorophyll

Leaf chlorophyll (Chl) concentration ( $\mu\text{mol m}^{-2}$ ) was estimated using a portable SPAD-502 meter (Minolta, Osaka, Japan). Readings were taken in the two youngest fully expanded leaves of each plant. Previously, a calibration curve was made for each rootstock (Table 1), by measuring the absorbance of chlorophyll with the SPAD, extracting the pigments from the same leaf area with 100% acetone in the presence of Na ascorbate (Abadía and Abadía, 1993) and measuring Chl spectrophotometrically according to Lichtenthaler (1987). SPAD readings were made at beginning and at the end of the experiment. To compare the changes in chlorophyll, the variation in Chl content was

Table 1

Relationship between leaf chlorophyll (Chl  $\mu\text{mol m}^{-2}$ ) and SPAD readings (S) for three citrus rootstocks (Troyer, Taiwanica and Swingle)

Rootstock	Calibration equation	$R^2$	$n$
Troyer	$\text{Chl} = 0.11S^2 + 2.90S - 14.21$	0.97	25
Taiwanica	$\text{Chl} = -0.08S^2 + 14.89S - 94.16$	0.93	20
Swingle	$\text{Chl} = 0.10S^2 + 2.19S + 4.28$	0.97	30

calculated as  $\{(\text{Chl}_e - \text{Chl}_b)/\text{Chl}_b\} \times 100$ , where  $\text{Chl}_b$  and  $\text{Chl}_e$  represents leaf chlorophyll concentrations at the beginning and at the end of the assay, respectively.

### 2.3. Growth parameters

At the end of the experiment, plants were separated into shoots (stems and leaves) and roots. The length of the shoot and the number of leaves on each plant were measured. Plant material was rinsed with tap water, distilled water containing a non-ionic detergent (10 mmol HCl  $\text{dm}^{-3}$ ) and finally three times in distilled water. The dry weight of each part was determined after drying at 70 °C. The ratio root dry weight: shoot dry weight was calculated for each rootstock and treatment to study the effect of Fe chlorosis on biomass allocation.

### 2.4. Mineral composition

Shoots (leaves and stems) were ground, ashed at 450 °C, and digested in an acidic solution (HCl 1 mol  $\text{dm}^{-3}$ ). The concentration of K, Ca, Mg, Mn, Zn, Cu, and Fe in the shoots was determined by atomic absorption spectrophotometry (Pye Unicam, Cambridge, UK) following standard methods (AOAC, 1990; Belkhdja et al., 1998b). Phosphorus was analysed colorimetrically by the molybdo-vanadate method at 420 nm.

### 2.5. Fluorescence parameters

At the end of experiment, chlorophyll fluorescence parameters ( $F_0$ : basal fluorescence;  $F_m$ : maximum fluorescence;  $F_v = F_m - F_0$ : variable fluorescence) were measured with a portable fluorimeter (Plant Efficiency Analyser, PEA, Hansatech Instruments Ltd., England) in the second fully developed leaf of each plant of 'Taiwanica' orange and 'Troyer' citrange rootstocks. After 20 min of dark adaptation, leaves were illuminated with a saturating pulse of 2100  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  for 5 s to induce fluorescence.

### 2.6. Statistical analysis

The means were compared by analysis of variance and by using the Duncan multiple range test at  $P \leq 0.05$ . For each rootstock, regression analysis was carried out between Fe concentration in shoots and chlorophyll concentration in leaves. The amounts of nutrients in shoot of each rootstock were also regressed in the relation of the level of Fe in solution. All the statistical analyses were done by using SAS software (SAS Institute, 1989).

### 3. Results

#### 3.1. Leaf chlorophyll

At the beginning of the experiment, the plants had no visual symptoms of iron deficiency. The Chl concentrations were similar for all treatments, and corresponded to  $409 \pm 26 \mu\text{mol Chl m}^{-2}$  in ‘Troyer’ citrange,  $436 \pm 5 \mu\text{mol Chl m}^{-2}$  in ‘Taiwanica’ orange, and  $243 \pm 46 \mu\text{mol Chl m}^{-2}$  in ‘Swingle’ citrumelo.

The leaves of ‘Troyer’ citrange and ‘Taiwanica’ orange rootstocks from the 0 and  $5 \mu\text{mol Fe dm}^{-3}$  treatments developed characteristic visual symptoms of Fe chlorosis after 2 weeks of growth that lasted until the end of the experiment. In both rootstocks the decreases in leaf Chl concentration were more pronounced in plants grown in the absence of Fe than in plants with  $5 \mu\text{mol Fe dm}^{-3}$  (Table 2). At the end of the experiment, the Chl concentrations of ‘Troyer’ citrange plants grown with 15 and  $20 \mu\text{mol Fe dm}^{-3}$  were greater than those of plants grown with  $10 \mu\text{mol Fe dm}^{-3}$ . In contrast, the Chl concentrations in ‘Taiwanica’ orange plants grown with 10, 15 and  $20 \mu\text{mol Fe dm}^{-3}$  were similar ( $524 \mu\text{mol Chl m}^{-2}$ ).

For ‘Swingle’ citrumelo rootstock the Chl concentration decreased in all treatments between the beginning and the end of the experiment, with the exception of the highest Fe level ( $20 \mu\text{mol Fe dm}^{-3}$ ). The plants from all treatments but the latter became chlorotic.

Table 2

Total chlorophyll ( $\mu\text{mol m}^{-2}$ ) at the beginning (Chl<sub>b</sub>) and at the end (Chl<sub>e</sub>) of the experiment, and the percentage of change in leaf chlorophyll between the beginning and the end of the experiment for each rootstock (Troyer, Taiwanica and Swingle) grown with different levels of Fe in solution in the presence of calcium carbonate

Fe ( $\mu\text{mol dm}^{-3}$ )	Total chlorophyll ( $\mu\text{mol m}^{-2}$ )		Relative variation (%)
	Chl <sub>b</sub>	Chl <sub>e</sub>	
<b>Troyer</b>			
Fe0	409 NS	79 e	–81 e
Fe5	409	214 d	–48 d
Fe10	409	614 c	50 c
Fe15	409	707 b	73 b
Fe20	409	793 a	94 a
<b>Taiwanica</b>			
Fe0	436 NS	167 c	–62 c
Fe5	436	303 b	–30 b
Fe10	436	520 a	19 a
Fe15	436	523 a	20 a
Fe20	436	530 a	22 a
<b>Swingle</b>			
Fe0	243 NS	55 c	–77 c
Fe5	243	75 c	–69 c
Fe10	243	86 c	–64 c
Fe15	243	132 b	–46 b
Fe20	243	279 a	15 a

Means followed by the same letter are not significantly different at  $P \leq 0.05$  (Duncan test). NS: not significant.

### 3.2. Growth analysis

Plants grown without Fe or with the lowest Fe level were smaller, with fewer leaves, and produced less dry matter (Table 3). However, the level of Fe in the nutrient solutions influenced the growth of each rootstock differently. In general, ‘Troyer’ plants grew better at the lower level of additional Fe ( $10 \mu\text{mol Fe dm}^{-3}$ ) than ‘Swingle’ plants ( $20 \mu\text{mol Fe dm}^{-3}$ ). ‘Taiwanica’ orange plants had an intermediate behaviour, with increased growth observed for  $10\text{--}15 \mu\text{mol Fe dm}^{-3}$ , depending on the parameter evaluated.

The relative increase in shoot dry weight of ‘Troyer’ citrange and ‘Taiwanica’ orange was greater than that of roots, but plants shoot and root dry weights of ‘Swingle’ citrumelo plants both increased fourfold from the control to  $20 \mu\text{mol Fe dm}^{-3}$ . In consequence, the ratio root dry weight: shoot dry weight decreased with increasing supply of Fe in ‘Troyer’ citrange and ‘Taiwanica’ orange rootstocks but remained constant in ‘Swingle’ citrumelo plants (Table 3).

### 3.3. Nutrient composition

The amount of P, K, Ca and Mg in shoots increased when iron was included in the nutrient solutions (Fig. 1). Linear relationships were established for some nutrients and rootstocks while in other cases a decrease in nutrient concentration was observed for the higher levels of Fe. In general, the largest increases were observed in the ‘Troyer’ plants and the smallest in the ‘Swingle’ plants.

Table 3

Shoot length, number of leaves, and root and shoot dry matter for three citrus rootstocks (Troyer, Taiwanica and Swingle) grown with different levels of Fe in the presence of calcium carbonate

Fe ( $\mu\text{mol dm}^{-3}$ )	Shoot length (cm)	Number of leaves	Dry weight (g)		
			Shoots	Roots	Root:shoot
<b>Troyer</b>					
0	7.4 b	9.5 b	0.3 b	0.3 b	1.06 a
5	7.4 b	11.7 b	0.4 b	0.3 b	0.79 b
10	13.5 a	16.1 a	1.0 a	0.6 a	0.60 b
15	12.3 a	15.4 a	0.9 a	0.6 a	0.66 b
20	11.9 a	14.4 a	0.9 a	0.6 a	0.63 b
<b>Taiwanica</b>					
0	7.5 b	8.6 c	0.1 b	0.1 c	1.10 a
5	7.2 b	11.1 c	0.4 b	0.2 bc	0.51 b
10	8.9 b	15.4 b	0.9 a	0.3 b	0.34 b
15	13.0 a	16.6 ab	1.2 a	0.4 a	0.37 b
20	12.4 a	19.2 a	1.0 a	0.3 b	0.29 b
<b>Swingle</b>					
0	5.7 b	6.9 b	0.2 b	0.1 b	0.60 NS
5	6.1 b	7.1 b	0.2 b	0.1 b	0.67
10	7.3 b	8.2 b	0.3 b	0.2 b	0.66
15	8.1 b	13.6 a	0.3 b	0.2 b	0.57
20	17.4 a	15.2 a	0.8 a	0.4 a	0.52

Means followed by the same letter are not significantly different at  $P \leq 0.05$  (Duncan test). NS: not significant. The ratio root dry weight:shoot dry weight is also presented.

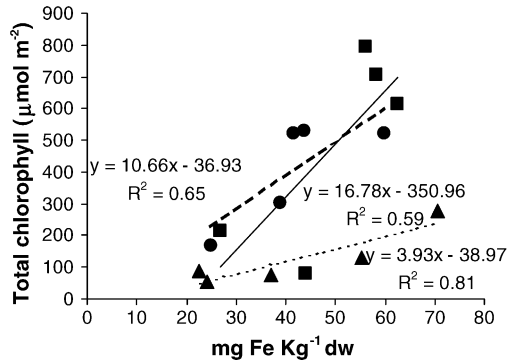


Fig. 1. Relationships between leaf chlorophyll ( $\mu\text{mol m}^{-2}$ ) of Troyer, Taiwanica and Swingle rootstocks and the Fe concentrations in the shoots ( $\text{mg kg}^{-1}$  dry weight). Only significant linear relations with  $P \leq 0.05$  are presented. Troyer (■, —), Taiwanica (●, ---), and Swingle (▲, ···).

The pattern of Fe accumulation in relation to increased supply was different in the three rootstocks (Fig. 2). In spite the linear adjustment, the greatest increases were obtained in ‘Troyer’ plants with a maximum content in shoots achieved at about  $10 \mu\text{mol Fe dm}^{-3}$ . The ‘Taiwanica’ plants showed a similar pattern for iron content, but in ‘Swingle’ plants there was little change in Fe content of the shoots until  $15 \mu\text{mol Fe dm}^{-3}$ , and thereafter the content increased rapidly. These results are similar to those reported for growth parameters.

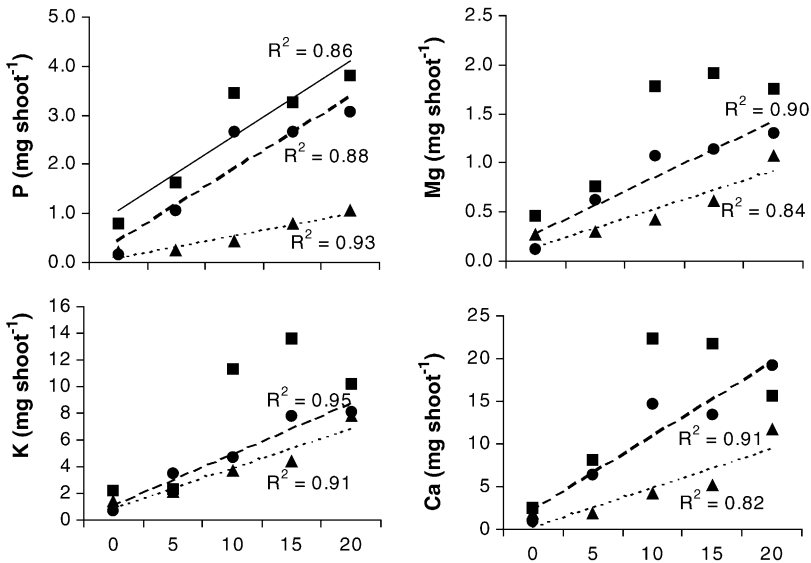


Fig. 2. Relationships between the contents of P, K, Ca and Mg in the shoots ‘Troyer’, ‘Taiwanica’ and ‘Swingle’ rootstocks and the level of Fe in solution (only significant linear relations with  $P \leq 0.05$  are presented;  $R^2$ : correlation coefficient). ‘Troyer’ (■, —), Taiwanica (●, ---), and ‘Swingle’ (▲, ···).

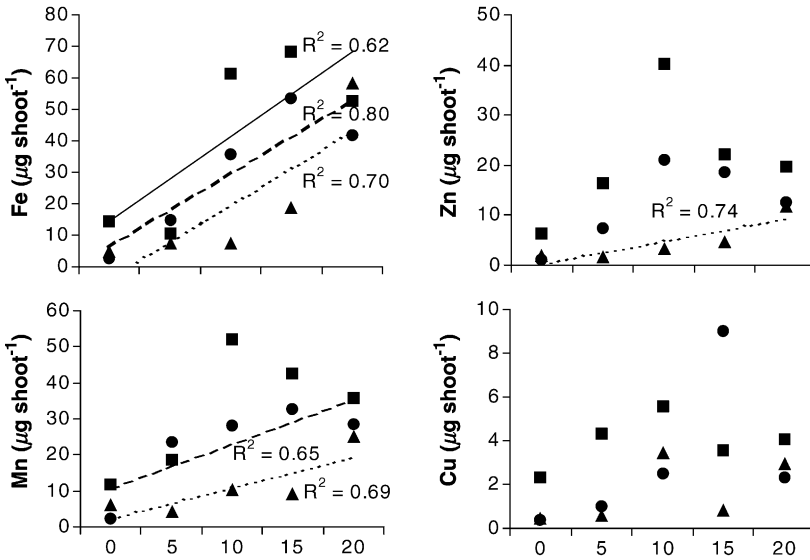


Fig. 3. Relationships between the contents of Fe, Zn, Mn and Cu in the shoots of ‘Troyer’, ‘Taiwanica’ and ‘Swingle’ rootstocks and the level of Fe in solution (only significant linear relations with  $P \leq 0.05$  are presented;  $R^2$ : correlation coefficient). ‘Troyer’ (■), Taiwanica (●, ---), and ‘Swingle’(▲, ···).

The pattern of Zn, Mn and Cu accumulation in ‘Troyer’ and ‘Taiwanica’ plants was in general similar, with the greatest contents reached when 10–15  $\mu\text{mol Fe dm}^{-3}$  was present (Fig. 3). For the ‘Swingle’ rootstock, the content of Zn and Mn tended to increase in shoots with increasing levels of Fe, while the pattern of Cu accumulation was unclear. The patterns of nutrient accumulation in roots were in general similar to those obtained in shoots (data not shown).

The increment of Fe concentrations in shoots was associated with an increase in leaf chlorophyll concentrations for all rootstocks, expressed by linear regressions (Fig. 1). However, for large Fe concentrations in shoots, the ‘Troyer’ and ‘Taiwanica’ plants showed more leaf Chl than ‘Swingle’ plants.

Table 4

Fluorescence parameters ( $F_0$ ,  $F_m$  and  $F_v/F_m$ ) measured at the end of experiment in Troyer ( $n = 29$ ) and Taiwanica ( $n = 25$ ) rootstocks grown with different Fe concentrations in the presence of calcium carbonate

Fe ( $\mu\text{M}$ )	Troyer			Taiwanica		
	$F_0$	$F_m$	$F_v/F_m$	$F_0$	$F_m$	$F_v/F_m$
0	844 NS	1785 b	0.48 b	1638 a	2375 d	0.32 c
5	921	2411 b	0.60 b	1282 b	2571 c	0.50 b
10	765	3502 a	0.78 a	889 c	2893 b	0.68 a
15	1013	3571 a	0.73 a	1039 c	3484 a	0.70 a
20	762	3530 a	0.78 a	1030 c	3645 a	0.72 a

$F_0$ : basal fluorescence;  $F_m$ : maximum fluorescence;  $F_v = F_m - F_0$ : variable fluorescence. Means followed by the same letter are not significantly different at  $P \leq 0.05$  (Duncan test). NS: not significant.



### 3.4. Fluorescence parameters

The values of  $F_0$  were similar in all treatments for ‘Troyer’ citrange plants, whereas for ‘Taiwanica’ orange  $F_0$  was lowest when Fe was applied at 10, 15 and 20  $\mu\text{mol Fe dm}^{-3}$  (Table 4). The  $F_m$  increased when Fe was supplied, reaching a maximum for 10  $\mu\text{mol Fe dm}^{-3}$  in ‘Troyer’ citrange and for 15  $\mu\text{mol Fe dm}^{-3}$  in ‘Taiwanica’ orange. For ‘Troyer’ citrange rootstock, the  $F_v/F_m$  ratios were significantly smaller in the absence of Fe and with 5  $\mu\text{mol Fe dm}^{-3}$  than in the other treatments. However, for ‘Taiwanica’ orange plants the  $F_v/F_m$  values were slightly higher in plants growing with 5  $\mu\text{M Fe}$  compared with plants grown without Fe.

## 4. Discussion

Several authors have classified Fe tolerance of citrus rootstocks supported on growth and chlorosis parameters of shoots (Hamzé et al., 1986; Sudahono et al., 1994; Byrne et al., 1995). They considered that ‘Troyer’ citrange and ‘Taiwanica’ orange plants had a similar degree of tolerance to Fe chlorosis, whereas the ‘Swingle’ citrumelo rootstock was more sensitive.

The three rootstocks had different chlorophyll concentration at the beginning of the experiment. Since there were no visual symptoms of iron deficiency at this point, the differences of chlorophyll content were probably related with foliar thickness. This fact may also explain the different calibration equations obtained for each rootstock.

The ‘Troyer’ citrange rootstock was more effective in overcoming the effects of the presence of bicarbonate since these plants grew more than plants of ‘Taiwanica’ orange and ‘Swingle’ citrumelo and accumulated a greater amount of Fe and chlorophyll in the shoots. To attain a significant vegetative growth, the latter rootstocks needed more Fe in solution compared with ‘Troyer’ citrange. The threshold values were 15  $\mu\text{mol Fe dm}^{-3}$  for ‘Taiwanica’ orange and 20  $\mu\text{mol Fe dm}^{-3}$  for ‘Swingle’ citrumelo.

The root:shoot ratio is normally used to assess the distribution of photoassimilates between shoots and roots (McDonald et al., 1996). The increase of root biomass in ‘Troyer’ citrange and ‘Taiwanica’ orange plants grown in the absence of Fe is a response already described for other species (Viti and Cinelli, 1993; Marschner et al., 1996; McDonald et al., 1996). It may be a response mechanism to the lack of Fe, associated with an increase in the number of secondary roots and the differentiation of transfer cells in the root apex (Bavaresco et al., 1994). These responses are normally shown by Fe-chlorosis tolerant genotypes (Socias i Company et al., 1995). Since ‘Swingle’ citrumelo rootstock had a constant root:shoot ratio, it is possible to admit a strong susceptibility of this genotype to this deficiency.

The values of chlorophyll may give information not only about the degree of chlorosis but also about the differential behaviour of genotypes to Fe chlorosis (Sudahono et al., 1994). It seems that ‘Troyer’ citrange maintained significantly greater chlorophyll contents compared with ‘Swingle’ citrumelo, suggesting that the latter is more sensitive to Fe chlorosis. This agrees with the results from other authors (Castle, 1987; Hamzé et al., 1986; Sudahono et al., 1994).

‘Troyer’ citrange and ‘Taiwanica’ orange plants showed Fe deficiency symptoms only for lower levels of Fe in solution, whereas ‘Swingle’ citrumelo plants showed chlorosis for all concentrations of Fe except  $20 \mu\text{mol Fe dm}^{-3}$ . These results indicate a differential response to inhibitory effects of bicarbonate ions in the absorption and translocation of Fe in plants (Mengel, 1995).

Based on growth parameters, and chlorophyll and nutrient contents, it became clear that ‘Troyer’ citrange and ‘Taiwanica’ orange rootstocks were more tolerant to Fe chlorosis than ‘Swingle’ citrumelo. Differences between ‘Troyer’ citrange and ‘Taiwanica’ orange were small though the former responded to iron more than the ‘Taiwanica’ orange rootstock. To further differentiate between these two rootstocks, fluorescence parameters were evaluated.

In vivo studies of fluorescence provide basic information about the photosynthetic apparatus, and in many situations there is an inverse relationship between photosynthetic activity and fluorescence of Chl (Morales et al., 2000). The efficiency of photosystem II is only slightly affected by iron deficiency in leaves of orange trees, sugar beet and pear (Morales et al., 1998; Pestana et al., 2001b). The decrease in  $F_v/F_m$  in ‘Troyer’ citrange was due to changes in the electron transport chain ( $F_m$  variation), whereas in ‘Taiwanica’ orange there were also structural modifications of PSII, particularly at the pigment level ( $F_0$  variation). It should be noted that increases in  $F_0$  could be originated from increases in the dark reduction of the plastoquinone pool, as shown recently for sugar beet and other species affected by Fe deficiency (Belkhdja et al., 1998a).

In conclusion, the evaluation of growth parameters may not be sufficient to evaluate tolerance to Fe chlorosis of citrus rootstocks. Using several physiological parameters, including chlorophyll and fluorescence parameters, it was possible to rank the three citrus rootstocks from ‘Troyer’ citrange as the most tolerant to ‘Swingle’ citrumelo as the most susceptible, and ‘Taiwanica’ orange with an intermediate behaviour.

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