



Phenomenon responses of two kiwifruit genotypes to calcareous and iron treatment in pot experiments

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Received: 2 November 2023 / Revised: 6 March 2024 / Accepted: 7 March 2024 / Published online: 8 January 2026
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Abstract

Screening Fe deficiency-tolerant genotypes is an environment-friendly and long-term effective strategy to cope with bicarbonate chlorosis stress in kiwifruit. We investigated the physiological response of two *Actinidia chinensis* var. *deliciosa* genotypes, cv. ‘Hayward’ and cv. ‘Tomuri’, when grown in the control solution (20 μ M FeEDDHA, Ct) and two iron-deprived conditions: (1) no Fe (0 μ M FeEDDHA, -Fe) and (2) induction of Fe deficiency by adding 10 mM HCO_3^- (Bic). After 45 treatment days, the -Fe and Bic plants exhibited leaf chlorosis symptomatology, as detailed by all the parameters, especially under the Bic conditions. The photosynthesis rate and the Fv/Fm time-course measurements reflected damage to leaf photosystems from early time points, especially in Hayward. The strategy I responses (reduction and acidification capacities) generally reduced, especially by the Bic treatment in both genotypes. However, Tomuri presented a more optimized system for mobilizing root Fe and maintaining photosynthetic activity with the Bic treatment by keeping proton and the Ferric Chelate Reductase levels better than Hayward. The most marked drop was for Chl in the Tomuri -Fe results from no Fe and biomass reduction, while the more attenuated decrease in Hayward depended only on ion nutrition. The results contribute by identifying potential responses in kiwifruit for Fe deprivation tolerance in calcareous soils.

Keywords *Actinidia chinensis* var. *Deliciosa* · Bicarbonate · Cv. hayward · Iron stress · Cv. tomuri

1 Introduction

Soils in the Mediterranean region are characterized by a high concentration of bicarbonate ions in both soils and irrigation water, and these lime-induced chlorosis conditions affect many annual crops and perennial plants. This is a major problem for most high-value fruit trees, especially in the Mediterranean basin or in other semiarid areas (Bavaresco et al. 2006). Thus it constitutes a dramatic limiting factor on both growth and production in kiwifruit vineyards in this region because this species is sensitive to lime-induced Fe chlorosis in fields and in vitro at a high-medium pH (Rombolà et al. 2002); Marino and Bertazza 1998).

As soil alkalinity reduces Fe accessibility and solubility by roots (Alizadeh et al. 2022), Fe deficiency leads to

interveinal yellowing on young leaves due to pigment synthesis disruption, smaller leaf size, less thickness and a lower branching rate, substantial losses of yield and fruit quality (unacceptable taste and short shelf life), and shorter vineyard longevity (Tagliavini and Rombola 2001; Yao et al. 2005). Consequently, bicarbonate-induced chlorosis has become one of the most crucial and limiting factors to influence the health and sustainable development of the kiwifruit industry in regions with this difficult soil type.

The high pH of these soils causes Fe precipitation as hydroxides (i.e. $\text{Fe}(\text{OH})_2$ and $\text{Fe}(\text{OH})_3$), and reduced activity of Fe chelate reductase (FC-R) at the cell membranes of the root cortical zone (Marschner and Römhild 1994). This enzyme is responsible for the reduction of Fe^{3+} to Fe^{2+} . The latter is the form that the roots of the plants characterized by “strategy I” can take up. These plants respond to Fe deficiency with protons (H^+) excretion lowering soil pH and activating the enzyme. However, this strategy does not suffice to counteract the effect of calcareous or alkaline soils. To prevent Fe deficiency from occurring, growers often apply synthetic Fe chelates to soil, as shown in the *Actinidia chinensis* Planch. var. *chinensis* ‘Hort16A’ cultivar,

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where additional Fe chelate applications in late summer and autumn increased average productivity by 56% and 23% in the 2 years following treatment, respectively (Thorp et al. 2021). However, these complexes are expensive and can cause groundwater pollution due to their high water solubility (Rombola et al. 2002). Therefore, alternative means for controlling Fe chlorosis should be developed to reduce costs and to improve environmental sustainability. Finally, rational concepts to deal with the problem focus on choosing the appropriate rootstock (Thorp et al. 2021).

Rootstocks have been widely used to control scion growth and development, to increase yield and quality (Singh et al. 2017; Gautier et al. 2019), and to improve scion resistance/tolerance to abiotic stresses (Kumar et al. 2017; Gautier et al. 2019) and biotic stresses (Guan et al. 2012) in graft-propagated plants. By focusing on Fe deficiency stress, growing suitable rootstocks that well tolerate soil alkalinity is a good strategy to overcome this problem, and is carried out in more fruit crops like citrus (Martínez-Cuenca, et al. 2013, 2016), wine grape (Assimakopoulou et al. 2016), olive and quince (Alcantara et al. 2003, 2012), and so on. Yet despite studies having been carried out in kiwifruit, the ideal rootstock has not yet been found, and the mechanisms involved in plant tolerance to calcareous soil conditions remain unclear.

In the kiwifruit family, *Actinidia chinensis* var. *deliciosa* (A.Chev.) A.Chev. is more tolerant to lime-induced Fe chlorosis than *Actinidia chinensis* Planch var. *chinensis*. In the first group, the Hayward cultivar is the most populated by growers and on international markets. It accounts for about 70% of all the kiwifruit worldwide due to its good export qualities (Huang 2016). Hayward plants are considered susceptible or highly susceptible to Fe deficiency depending on the propagation method (micropropagated or cuttings, respectively), and are also sensitive to other abiotic stresses like drought and waterlogging (Tagliavini and Rombola 2001). However, Hayward together with Shaanmi cultivars are harder to chlorosis than other Chinese cultivars like Qinmei (Xufeng et al. 2002), while the Italian D1 Bruno cultivar is less susceptible than Hayward (Vizzotto et al. 1999; Tagliavini and Rombola 2001). As dioecious plants, today the more frequently accompanying male vines is the Tomuri cultivar, a late-flowering male cultivar, which normally flowers from mid- to late May and is used as the main pollinizer for Hayward (Zhu 2002). However, information about its response to abiotic stresses in this cultivar is lacking (Prado et al. 2006; Huang 2016).

Given these considerations, the objectives of this work were to: (1) evaluate the physiological response of two kiwifruit cultivars (Hayward and Tomuri) when subjected to Fe deprivation stress conditions when Fe is absent in the culture solution or bicarbonate is present: (2) compare some items of the strategy I mechanism in both cultivars for the

ultimate purpose of supplying new information about their tolerance response to this stress. The overall goal is to provide growers with more reliable information on using these plants as potential rootstocks so they can deal with the problem in their orchards.

2 Materials and methods

2.1 Plant material and growth conditions

The 12-month-old *Actinidia chinensis* var. *deliciosa* cv. Hayward and *Actinidia chinensis* var. *deliciosa* cv. Tomuri plants were obtained from the Vivers Hernandorena S.L. nursery (Carles Salvador str, s/n, Carlet, Spain). Plants were grown individually in opaque plastic 4-L pots filled with sand. Plants were irrigated twice weekly with the following basal nutrient solution (pH 6.0) at half strength: 5 mM $\text{Ca}(\text{NO}_3)_2$, 1.4 mM KNO_3 , 2 mM MgSO_4 , 0.6 mM H_3PO_4 , 20 μM Fe-EDDHA, 7.6 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 50 μM H_3BO_3 , 0.50 μM MoO_3 and 54 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$. Plants were acclimated for 2 weeks before experiments began under glasshouse conditions (26–28/16–18 °C, 70–80% and a 16-hour photoperiod).

2.2 Iron stress treatments

After the preconditioning time, plants were selected based on size uniformity (2–3 shoots, 100 cm mean total plant height) and transplanted individually to opaque plastic 5-L pots filled with coarse sand. Then plants were separated into three groups and irrigated with the following basal nutrient solution (BSN): 3 mM $\text{Ca}(\text{NO}_3)_2$, 3 mM KNO_3 , 2 mM MgSO_4 , 2.3 mM H_3PO_4 , 46.25 μM H_3BO_3 , 54.4 μM $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 7.65 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.55 μM MoO_3 and 0.5 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, supplemented or not with o, o-FeEDDHA and HCO_3^- , to establish three treatments as follows:

Control (Ct): BSN+20 μM o, o-FeEDDHA+0 mM HCO_3^-

Treatment 1 (-Fe): BSN+0 μM o, o-FeEDDHA+0 mM HCO_3^-

Treatment 2 (Bic): BSN+20 μM o, o-FeEDDHA+10 mM HCO_3^-

pH was adjusted to 7.5 with 1 M KOH or 1 M H_2SO_4 . Plants were randomized over the experimental area and a row of plants, not included in the experiment, was placed around the perimeter as a buffer row. Plants (six of each variety and treatment) were maintained for 45 days under the same

previously described glasshouse conditions. At the end of the experiment, plants were separated into leaves, stems and roots (coarse and fibrous fractions), rinsed with tap water and finally rinsed 3 times in distilled water. Each plant fraction was randomized, and one sample was kept to determine biomass dry weight and mineral concentration. Roots were rinsed once with 0.05 M HCl for 3 min to remove any Fe bound to cell walls, and then twice with distilled deionized water. A second sample of leaves was frozen and kept at -80°C until further used as fresh material.

2.3 Growth parameters

Some growth parameters were measured in the treated plants (six plants for variety and treatments) at the end of the experiment (45 days). The dry weight (DW, in g) of each plant fraction (leaf, stem, coarse root and fibrous root) was determined after drying at 70°C for 2 days to constant weight. Leaf number and leaf area were measured, and the root/shoot ratio was calculated.

2.4 Chlorophyll concentration

The leaf chlorophyll (Chl) concentration at the end of the experiment (45 days) was measured by a spectrophotometer (Lambda 25, PerkinElmer, Shelton, CT, USA) according to Moran and Porath (1980). The leaf freeze sample was ground with a ball mill and 20 mg were incubated in 6 mL n, n-dimethylformamide at 4°C for 24 h, and centrifuged for 15 min at 6000 g and 4°C . The supernatant was left for 1 h in the presence of Na_2SO_4 and its absorbance was measured at 647 and 664 nm (Lambda 25, PerkinElmer, Shelton, CT, USA). Three measurements were taken per treated plant. The average value of three measures was considered to be representative of each individual plant.

2.5 Fluorescence parameters

The maximum efficiency of photosystem II (Fv/Fm) under dark-adapted conditions was measured at 0, 15, 30 and 45 days after treatment in all the plants from 12:30–14:00 (UT+01:00) under saturating light conditions ($1000\ \mu\text{mol m}^{-2}\text{s}^{-1}$) with a portable fluorimeter (Handy PEA, Hansatech instruments, King's Lynn, UK). Measurements were taken on two fully expanded leaves per plant (the 5th–6th leaves from the apex) from all the plants. The average value of the two leaves was considered to be representative of each individual plant.

2.6 Gas exchange parameters

The net CO_2 assimilation rate (A_N , $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2}\text{ s}^{-1}$), substomatal CO_2 concentration (C_i $\mu\text{mol CO}_2\text{ mol}^{-1}$) and stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2}\text{ s}^{-1}$) of the single attached leaves were measured in the greenhouse between 10:00 h and 11:30 h on a sunny day, which allowed measurements to be taken under relatively stable conditions. Photosynthetically active radiation (PAR) on the leaf surface was adjusted to a photon flux density of $1000\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$. A portable closed gas exchange chamber (CIRAS-2, PP-systems, Hitchin, UK) was used for measurements. Leaf laminae were fully enclosed inside a PLC 6 (U) universal leaf autocuvette in a closed-circuit model and were kept at $25\pm 0.5^{\circ}\text{C}$ with a leaf-to-air vapor deficit of about 1.7 Pa. The air flow rate applied through the cuvette was 200 mL min^{-1} . Measurements were taken at 0, 15, 30 and 45 days after treatment on two fully expanded leaves (the 5th–6th leaves from the apex) on all the plants. The average value of the two leaves was considered to be representative of each individual plant. In addition, parameters A_N/C_i and A_N/E were calculated as instantaneous carboxylation and water use efficiency, respectively, at the end of the experiment.

2.7 Total Fe concentration

The total Fe concentration ($\mu\text{g Fe g}^{-1}\text{ DW}$) at the end of the experiment (45 days) was determined in the leaves and fibrous root fractions. Thus 0.5 g of the dried material was burnt in a muffle furnace for 12 h at 550°C . Subsequently, Fe was extracted with 2% nitric acid (Hiper-pur Panreac, $\text{Fe} < 1\text{ ppb}$) in an ultrasonic bath (Fungilab S.A., SantFeliu de Llobregat, Barcelona, Spain) for 30 min at 40°C . The total Fe concentrations were measured by an atomic absorption spectrometer (AAS PerkinElmer AAnalyst 200, Waltham, Massachusetts, USA). All the analyses were carried out with ultrapure water (Ultra Pure Water Systems, Milli QPlus). The total ion content (μg) was also calculated.

2.8 Fe chelate reductase (FC-R) activity

FC-R activity from the root system at the end of the experiment (45 days) was determined by measuring the formation of Fe^{2+} and the BPDS complex from Fe^{3+} -EDTA (Chaney et al. 1972).

Ten tips of apical root segments (about 5–8 mm long, 0.020 g FW approx.) were incubated in triplicate for all the plants. The average value of the three measurements was considered representative of each individual plant. Tips were rinsed with 0.2 mM $\text{CaSO}_4\cdot 2\text{H}_2\text{O}$ for 5 min and then incubated in 10 mL of fresh nutrient solution, supplemented

with 0.3 mM BPDS (Acros Organics, New Jersey, USA) and 100 μM Fe^{3+} -EDTA (Sigma-Aldrich, St Louis, MO, USA). The assay solution pH was previously adjusted to pH 5.5 with 5 mM MES-NaOH (morpholineethanesulfonic acid) (Panreac, Barcelona, Spain). The beaker was completely covered with aluminum foil to exclude light at 23 °C. After 1–6 h of incubation, aliquots were removed hourly and absorbance at 535 nm was determined by a spectrophotometer (Lambda 5, PerkinElmer, Shelton, CT, USA). BPDS forms a stable water-soluble red complex with Fe^{2+} and a weak complex with Fe^{3+} . The amount of reduced Fe was calculated by the Fe^{2+} -(BPDS)₃ complex concentration using an extinction coefficient of 22.14 $\text{mM}^{-1}\text{cm}^{-1}$ and was expressed as $\mu\text{mol Fe}^{2+}$ reduced g^{-1} root FW h^{-1} .

2.9 Acidification of root media

The quantitative determination of medium acidification at the end of the experiment (45 days) was determined in the roots of all the plants. Plants were transferred to beakers, which contained 1 L of nutrient solution either with 20 μM FeEDDHA for the Ct and Bic plants or without FeEDDHA for the -Fe plants. Solutions were adjusted to pH 7 with 5 mM NaOH (Panreac, Barcelona, Spain). Beakers were completely covered with aluminum foil to exclude light, and solutions were bubbled continuously with air. Plants were left in the greenhouse under the same growing conditions as they were cultivated. Medium acidification was determined by measuring pH changes in the nutrient solution. pH values were recorded at different times over an 8-hour period using a pHmeter (Consort C531, Turnhout, Belgium) and the proton extrusion from both cultivars. The three treatments were determined 24 h later.

2.10 10 statistical analyses

For the statistical analyses, values were the means and the standard deviations of six independent plants per treatment. Data distributions were checked for normality. Statistical analyses were carried out using analyses of variance (ANOVA) with Statgraphics Plus, version 5.1 (Statistical Graphics, Englewood Cliffs, NJ, USA). When the ANOVA showed a statistical effect, means were separated by the multiple comparison procedure to determine which means were significantly different from one another following Fisher's least significance difference (LSD) method at the 95% confidence level.

One correlation analyses was completed among the measured responses, in which the individual samples of each accession were subjected to linear regression and correlation coefficients (r).

Furthermore, a principal component analysis (PCA) was run for the standardized values using pairwise Euclidean distances among the responses means to determine any relations between the treated genotypes. The extracted eigenvalues, and the relative and cumulative proportions of the total variance explained by the first three components, were calculated. Two-dimensional (2D) scatter plots (the first vs. the second and the first vs. the third principal components; PCs) were prepared based on a distance matrix for the PCs to visualize the relation that explained traits. For the PCA analysis, the data pertaining to the six treatments were considered together.

3 Results

3.1 Development of chlorosis and plant growth

Forty-five days after plants were exposed to the Fe deficiency treatment, they developed chlorosis symptoms on apical leaves (Fig. 1A). The color of the foliage from both cultivars irrigated with treatments -Fe and Bic ranged from pale green to bright yellow, while leaf veins were still a darker green (interveinal chlorosis). As a general trend, the leaves and the root system (Fig. 1B) in the -Fe and Bic plants were visually similar to the Ct ones.

The plants irrigated with Ct solutions showed differences in the plant growth parameters (Table 1). Under the Ct conditions, the Tomuri plants presented higher growth parameter values than the Hayward plants for leaf number, leaf DW, stem DW and coarse root DW (146.7%, 49.0%, 76.6% and 55.1%, respectively).

Fe deficiency affected the plant growth parameters, albeit with some differences between cultivars. The leaf number in the Tomuri -Fe cultivar was lower by 25.8% compared to the Tomuri Ct, while no significant differences were found between Hayward -Fe and Hayward Ct (Table 1). Fe deficiency induced by adding bicarbonate (Bic) reduced this parameter in both cultivars compared to the Ct plants (33.3% and 24.2% in Hayward Bic and Tomuri Bic, respectively). Leaf surface was similar between Ct cultivars, but Fe deficiency decreased this parameter in both kiwifruit plants, with no differences between the -Fe and Bic treatments. Leaf DW reduced in Tomuri -Fe and Tomuri Bic (30.6% and 14.2%, respectively) compared to the Tomuri Ct. Bic treatment also reduced leaf DW in the Hayward plants (31.4%). Stem DW was significantly reduced by Fe deficiency (-Fe) in Tomuri (30.2% decrease), but not in Hayward. The Bic treatment markedly reduced stem DW in both cultivars (76.2% and 80.0% in Tomuri Bic and Hayward Bic, respectively). Coarse root DW was not affected by either the -Fe or Bic treatment in the Tomuri cultivar, but was significantly

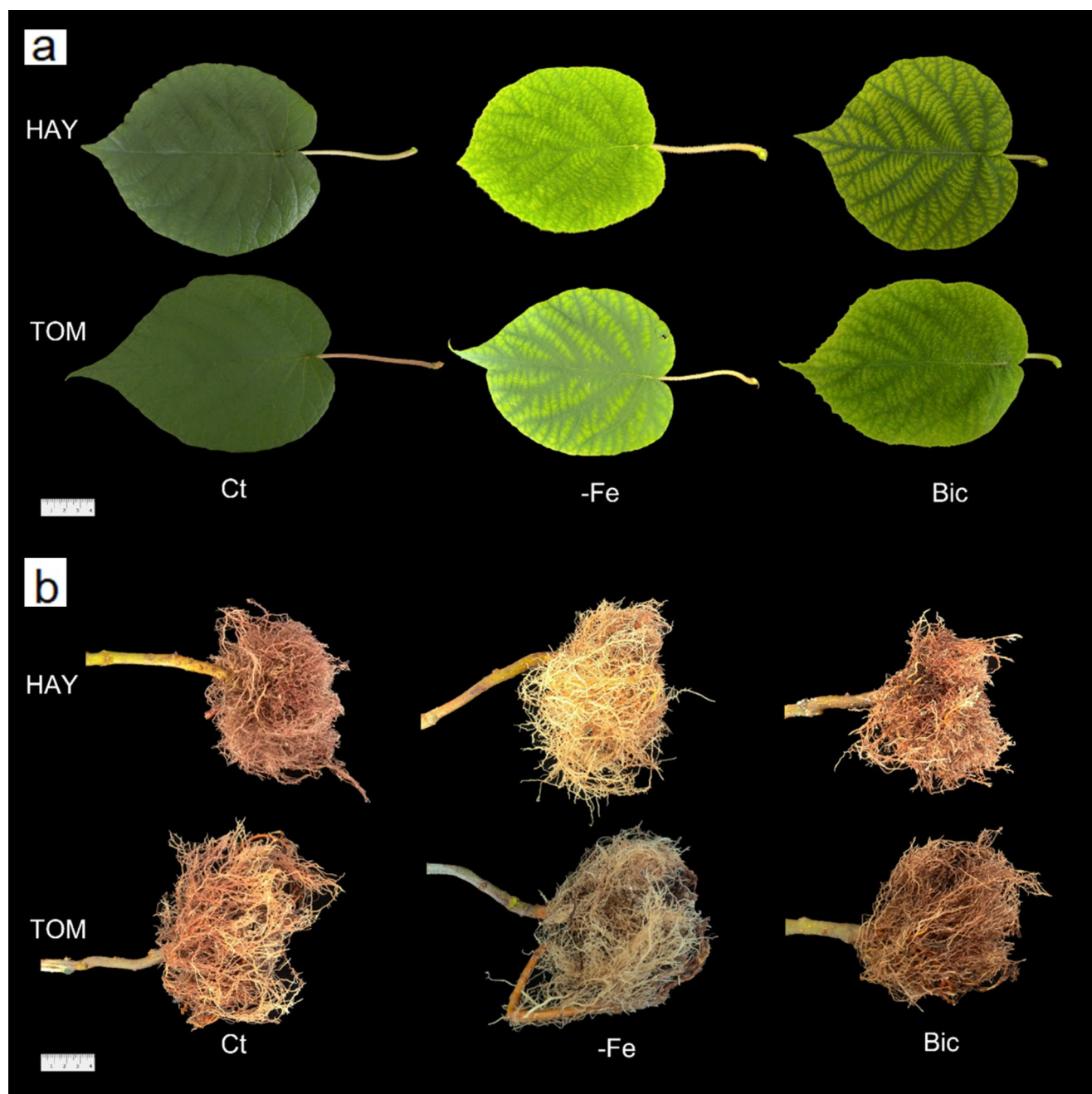


Fig. 1 Visual symptoms in the fully expanded leaves (**a**) and root system (**b**) in the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days in nutrient solutions with or with-

out 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO_3 (Bic)

reduced in Hayward -Fe and Hayward Bic compared to the Hayward Ct (30.1% and 31.4%, respectively). Fibrous root DW was not affected by either the -Fe or Bic treatment in the Tomuri or Hayward cultivars.

The root/shoot ratio (Table 1) was similar between the Ct plants (0.176 ± 0.14). No Fe in the nutrient solution significantly increased this parameter in the Tomuri -Fe plants (34.9%), but not in the Hayward -Fe ones compared to the Ct plants. The root/shoot ratio markedly rose in both

cultivars with the addition of Bic (by 86.7% and 64.5% in Tomuri Bic and Hayward Bic, respectively).

3.2 Chlorophyll concentration

The -Fe treatment reduced Chl a by 52.7% and 68.9% in Hayward -Fe and Tomuri -Fe, respectively (Fig. 2A). The lowest Chl a value in Hayward was for the Bic treatment (68.8% and 33.9% lower than Hayward Ct and Hayward

Table 1 Leaf number, leaf area (cm²), leaf, stem, coarse and fibrous root biomass (g DW) in the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days in nutrient solutions with or without 20 μ M o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μ M o, o-FeEDDHA supplemented with 10 mM NaHCO₃ (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). In each row, for the comparison of means, an ANOVA, followed by the Fisher’s least significance difference (LSD) test calculated at the 95% confidence level, was performed. The different letters in the same row indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and Ns (non-significant). DW: dry weight

	ANOVA																	
	Hayward						Tomuri											
	Ct	-Fe	Bic	Ct	-Fe	Bic	Ct	-Fe	Bic	Ct	-Fe	Bic						
Leaf number	49.3	c	31.3	c	d	115.7	a	85.9	b	87.8	b	***	C	***	T	***	CxT	**
Leaf area (cm ²)	166.4	a	141.8	a	b	188.7	a	152.5	b	158.7	b	***	C	**	***		ns	
Leaf DW (g)	17.1	b	14.2	bc	11.7	c	25.4	a	17.6	b	21.8	a	***	C	***	**	ns	
Stem DW (g)	16.7	c	13.2	c	3.4	d	29.6	a	20.6	b	7.0	d	***	C	***	***	**	
Coarse root DW (g)	4.9	b	3.4	c	3.4	c	7.6	a	7.0	a	7.0	a	***	C	***	**	ns	
Fibrous root DW (g)	1.3	b	1.2	b	1.2	b	1.5	ab	1.5	ab	1.9	ab	**	C	**	ns	ns	
Root/shoot ratio	0.19	bc	0.17	c	0.31	a	0.17	c	0.22	b	0.31	a	ns	C	ns	***	*	

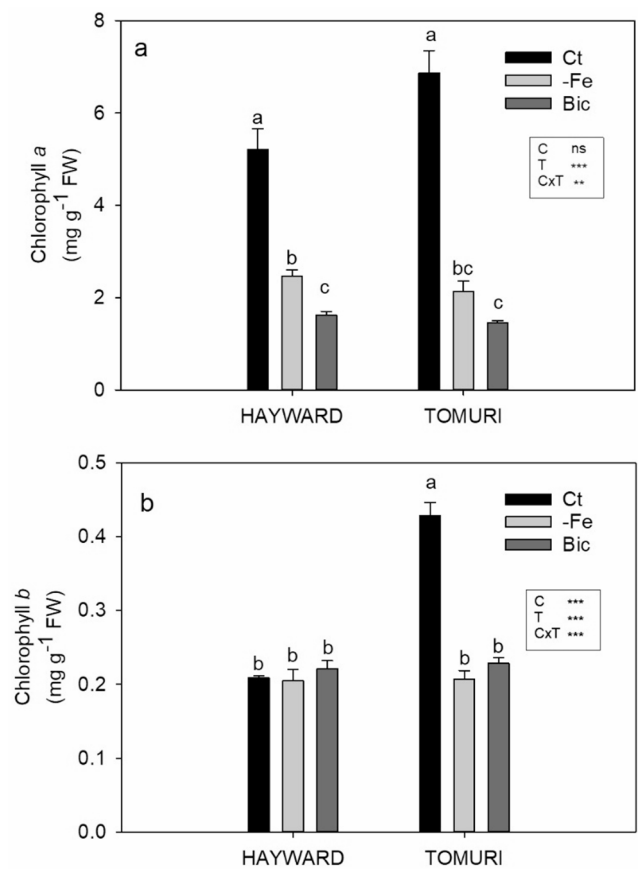


Fig. 2 Chlorophyll *a* (a) and chlorophyll *b* (b) concentrations (mg g⁻¹ FW) in the fully expanded leaves from the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days in nutrient solutions with or without 20 μ M o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μ M o, o-FeEDDHA supplemented with 10 mM NaHCO₃ (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). In each row, for a comparison of means, an ANOVA followed by the Fisher’s least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant). FW: Fresh weight

-Fe, respectively). The Bic treatment also lowered the Chl *a* concentration in the Tomuri cultivar (78.9% reduction) *versus* Tomuri Ct, but without differences with the Tomuri -Fe plants. The Ct plants showed differences in the Chl *b* concentration (201.0% higher in Tomuri Ct than in Hayward Ct, Fig. 2B). The Fe deficiency treatments reduced Chl *b* in the Tomuri cultivar by 75.1% and 50.4% in Tomuri -Fe and Tomuri Bic, respectively, but no effect was observed in Hayward -Fe and Hayward Bic.

3.3 Fluorescence parameters

The maximum quantum yield values for PS II (Fv/Fm, Fig. 3A) in the young leaves of the Fe-sufficient plants (Ct)

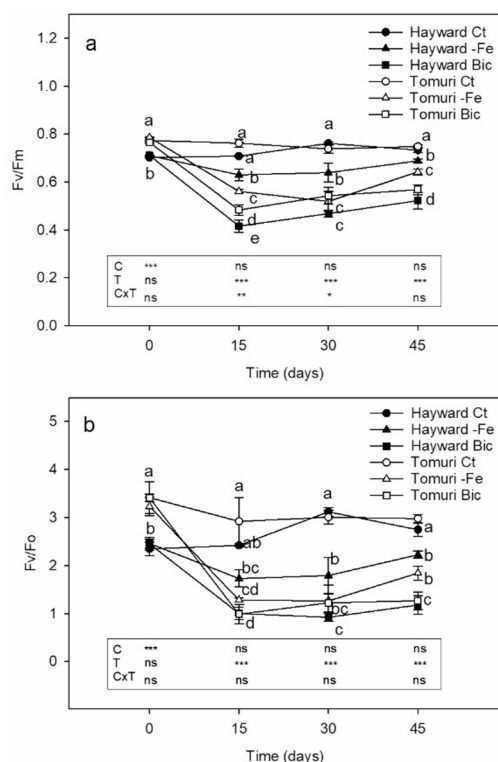


Fig. 3 Chlorophyll fluorescence parameters: Fv/Fm (a) and Fv/Fo (b) in the fully expanded leaves from the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 0, 15, 30 and 45 days in nutrient solutions with or without 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO₃ (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). On each date, for a comparison of means, an ANOVA followed by the Fisher’s least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters on the same date indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant). DW: Dry weight

were higher in Tomuri than in Hayward (0.76 ± 0.03 and 0.70 ± 0.01 , respectively). Fifteen days after treatment, both cultivars had a lower Fv/Fm parameter value in the leaves from the -Fe and Bic treatments related to Ct (11.0% and 41.2%, respectively in Hayward, and 26.4% and 36.6%, respectively in Tomuri). The same pattern was observed after the 30- and 45-day treatments. A general trend was noted for the lowest Fv/Fm values observed in the plants irrigated with the Bic treatment, and independently of measurement time.

The variable to initial fluorescence ratio (Fv/Fo, Fig. 3B) was higher in Tomuri Ct than in Hayward Ct (3.32 ± 0.16 and 2.42 ± 0.04 , respectively). Fifteen days after treatment, the Hayward plants had a lower Fv/Fo ratio in the Bic leaves (58.7% decrease), but not in the -Fe ones compared to the Ct. This parameter in the Tomuri plants was significantly lower than in Tomuri Ct in both Fe chlorosis treatments (56.1%

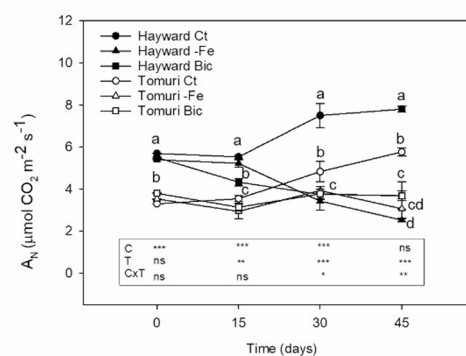


Fig. 4 Net CO₂ assimilation rate (A_N, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in the fully expanded leaves from the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 0, 15, 30 and 45 days in nutrient solutions with or without 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO₃ (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). On each date, for a comparison of means, an ANOVA followed by the Fisher’s least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters on the same date indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant)

and 66.2% decrease in -Fe and Bic, respectively). When Fv/Fo was measured at 30 and 45 days after treatment, it was lower in both cultivars and for both Fe-deprived conditions. The lowest Fv/Fo values were recorded in the plants irrigated with the Bic treatment (between 57.2% and 70.4%), while this drop ranged between 19.0% and 57.9% in the -Fe plants.

3.4 Gas exchange parameters

The net CO₂ assimilation rate values (A_N, Fig. 4) in the young leaves of the Fe-sufficient plants (Ct) were higher in Hayward than in Tomuri ($5.53 \pm 0.15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $3.54 \pm 0.25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively). Fifteen days after treatment, the A_N value of Hayward Bic had lowered by 21.7% versus Hayward Ct, while no effect was observed for Hayward -Fe, Tomuri -Fe and Tomuri Bic compared to their Ct plants. Thirty days after treatment, A_N had reduced in both cultivars, but more markedly in Hayward (41.3% and 50.7% reduction in Hayward -Fe and Hayward Bic, respectively) than in Tomuri (18.8% and 22.9% reduction in Tomuri -Fe and Tomuri Bic, respectively). The same pattern was observed 45 days after treatment. A more marked drop in Hayward (67.9% and 52.6% reduction) than in Tomuri (46.2 and 35.4% reduction) was obtained for the -Fe and Bic plants, respectively, in relation to their Ct plants.

The instantaneous carboxylation efficiency parameter (Fig. 5A) calculated at the end of the experiment, and estimated as the A_N/C_i ratio, was 30.4% higher in Hayward Ct than in Tomuri Ct (0.030 and 0.023 values, respectively).

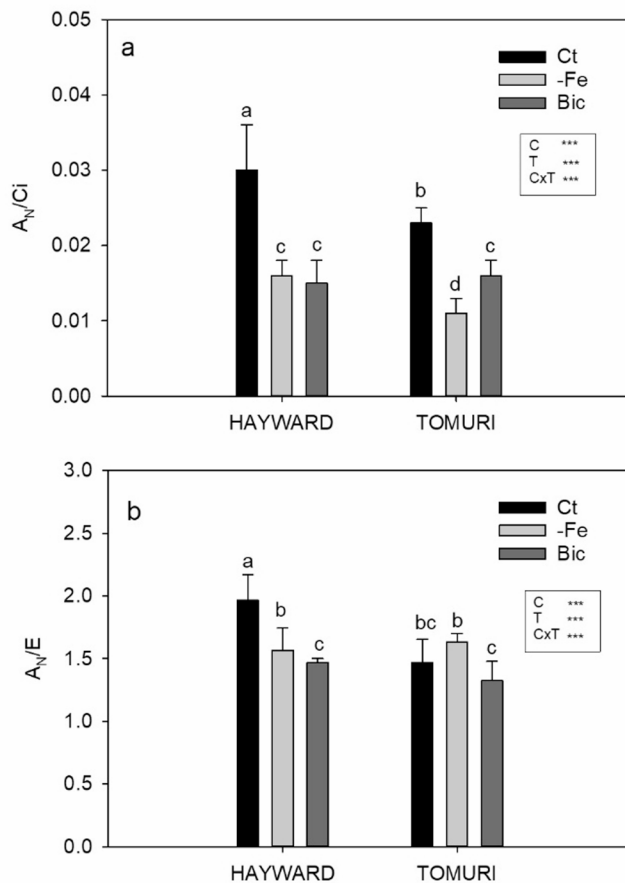


Fig. 5 Instantaneous carboxylation efficiency (A_N/C_i) (a) and instantaneous water use efficiency (A_N/E) (b) in the fully expanded leaves from the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days in nutrient solutions with or without 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO_3 (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). For a comparison of means, an ANOVA followed by the Fisher’s least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant)

Both the Fe-deficiency treatments similarly lowered the A_N/C_i ratio in Hayward -Fe and Hayward Bic (between 50.0% and 46.7%, respectively), while the A_N/C_i value dropped more in the Tomuri -Fe plants (52.2% decrease) than in the Tomuri Bic (30.4% decrease) ones.

The instantaneous water use efficiency parameter (A_N/E , Fig. 5B), estimated as the CO_2 assimilation (A_N) to transpiration (E) ratio, was 34.1% higher in the Hayward plants than in the Tomuri ones grown under the Ct conditions. Both the -Fe and Bic treatments lowered A_N/E in the Hayward plants (20.3% and 25.4% decrease, respectively), but not in Tomuri -Fe or Tomuri Bic compared to the Ct plants.

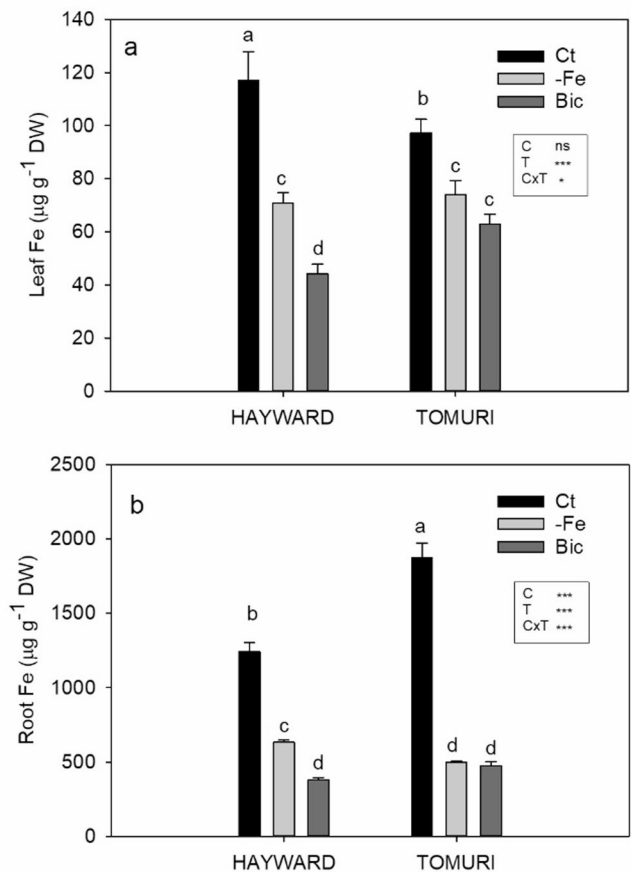


Fig. 6 Total Fe concentration ($\mu\text{g g}^{-1}$ DW) in the fully expanded leaves (a) and root system (b) in the ‘Hayward’ and ‘Tomuri’ kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days in nutrient solutions with or without 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO_3 (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). For a comparison of means, an ANOVA followed by the Fisher’s least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant). DW: Dry weight

3.5 Total Fe concentration

The Fe concentration in the Hayward Ct plant leaves was 20.3% higher than in the Tomuri Ct ones (Fig. 6A). The -Fe treatment lowered the Fe concentration in this organ by 39.4% and 23.7% in Hayward -Fe and Tomuri -Fe, respectively. The most marked drop in the Fe concentration in leaves was recorded for the Hayward Bic plants (62.2% reduction compared to Hayward Ct), while no significant differences were found between the Tomuri -Fe and Tomuri Bic values.

The total Fe concentration in the Hayward Ct plant roots was 50.8% lower than in the Tomuri Ct ones (Fig. 6B). Both the -Fe and Bic treatments lowered the Fe concentrations

in the roots from both cultivars. This reduction was statistically different between treatments in the Hayward cultivar (49.0% and 69.3% in Hayward -Fe and Hayward Bic, respectively, compared to the Ct plants), while a similar drop was recorded for the Tomuri plants with both the Fe-deficient treatments (around 74.0%).

3.6 FC-R activity

The data from Fig. 7A show that plants' Fe^{3+} reducing capacity in roots significantly differed. Under the Ct conditions, Tomuri presented greater FC-R activity than Hayward (68.2% increase). The -Fe treatment lowered the FC-R value in both cultivars compared to the Ct plants (60.1% and

48.7% reduction in Hayward -Fe and Tomuri -Fe, respectively). The Bic conditions increased enzymatic activity in the Hayward Bic plants (111.2% increase), but not in the Tomuri Bic ones, in relation to their corresponding Ct.

3.7 Acidification of root media

The determination of root acidification capacity, measured as proton extrusion, gave differences between the Actinidia cultivars (Fig. 7B). The Hayward Ct plants presented higher extrusion capacity than Tomuri Ct (35.4% increase). Proton extrusion significantly reduced in the Hayward -Fe plant roots (16.9% reduction) versus the Hayward Ct ones, while this response was not observed in the Tomuri cultivar. The Bic treatment lowered this parameter in both cultivars (86.7% and 90.0% in Hayward Bic and Tomuri Bic, respectively).

3.8 Correlation between physiological responses

Correlation analyses were carried out to estimate the relation between the physiological responses in the plants of both genotypes grown according to the three treatments (Table 2). The pairwise coefficients showed a positive correlation and statistical significance for 36 pairs of traits of the 105 studied ones. The most representative positive relations were observed among pigments, fluorescence parameters, A_N , Fe concentration and H^+ efflux. Leaf number correlated with stem and coarse root DW, Chl b and root Fe. FC-R activity positively correlated with leaf DW and the root/shoot ratio, and was related to fluorescence and the Fe concentration.

Statistically significant negative correlations for pairs of traits were also determined in nine of the 105 studied ones. The closest negative relations were for the root/shoot ratio vs. six traits (Fv/Fm, Fv/Fo, Chl a, leaf Fe, root Fe and H^+ -efflux) and for FC-R activity vs. H^+ -efflux.

3.9 PCA analysis

The PCA analysis revealed four PCs whose eigenvalues were above 1 (Table S2, Fig. S1). The distribution of PC1, PC2 and PC3, which respectively accounted for 53.35%, 19.43% and 14.77% (Table S2), of the total variation, are shown. Low correlation coefficients, with values under 0.150, were not presented.

The projection on the PCA plot showed clear differences between genotypes. The strongest positive relations in PC1 were observed with stem DW, Chl a, Fv/Fm, Fv/Fo, leaf Fe and root Fe (Fig. S2), with a negative one for the root/shoot ratio. Therefore, the plants with the highest stem DW, chlorophyll and Fe concentration values were placed to the

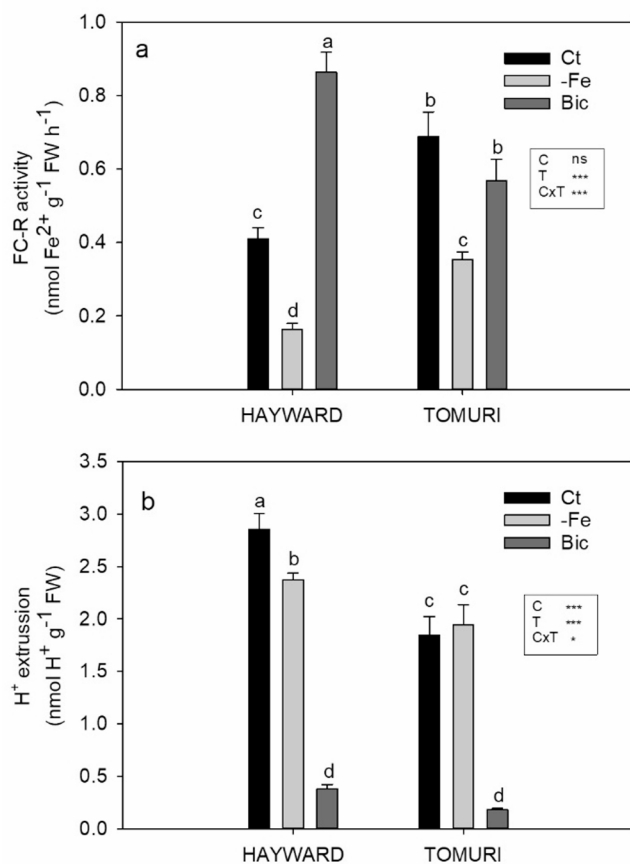


Fig. 7 Reduction capacity (a) measured as ferric chelate reductase (FC-R) activity (as $\text{nmol Fe}^{2+} \text{g}^{-1} \text{FW h}^{-1}$) and acidification capacity (b) measured as proton extruded (in $\text{nmol H}^+ \text{g}^{-1} \text{FW}$) in the roots of the 'Hayward' and 'Tomuri' kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 0, 15, 30 and 45 days in nutrient solutions with or without 20 μM o, o-FeEDDHA (Ct and -Fe, respectively) and 20 μM o, o-FeEDDHA supplemented with 10 mM NaHCO_3 (Bic). The values are the means $\pm$ SD of six independent plants ($n=6$). On each date, for a comparison of means, an ANOVA followed by the Fisher's least significance difference (LSD) test, calculated at the 95% confidence level, was performed. The different letters on the same date indicate significant differences between the distinct cultivars (C) and treatments (T). Levels of significance are represented by $P<0.05$ (*), $P<0.01$ (**), $P<0.001$ (***) and ns (non-significant). FW: Fresh weight

Table 2 Linear correlation coefficient (r) and its significance between physiological responses in the 'Hayward' and 'Tomuri' Kiwifruit (*Actinidia chinensis* var. *deliciosa*) irrigated for 45 days under the Fe-deficient conditions. ***, **, * indicate significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$ values for r . DW: dry Weight, chl: Chlorophyll, A_N : net CO_2 assimilation rate, FC-R: Fe chelate Reductase, H^+ : proton

TRAITS	Leaf number	Leaf DW	Stem DW	Coarse root DW	Fibrous root DW	Root/Shoot	Chl a	Chl b	Fv/Fm	Fv/Fo	A_N	Leaf Fe	Root Fe	FC-R	H^+ efflux
Leaf number															
Leaf DW		-0.240	0.665 **	0.837 ***	0.014	-0.2410	0.412	0.693 **	0.354	0.330	-0.001	0.255	0.515 *	0.017	-0.010
Stem DW			-0.237	-0.321	-0.133	0.1507	-0.166	0.119	-0.349	-0.253	-0.251	-0.466*	-0.131	0.491 *	-0.399
Coarse root DW				0.553 *	-0.139	-0.7464 ***	0.691 **	0.604 **	0.775 ***	0.770 ***	0.275	0.593 **	0.702 **	-0.281	0.585 *
Fibrous root DW					0.059	0.0355	0.182	0.468	0.125	0.095	0.038	0.068	0.254	0.045	-0.170
Root/Shoot						0.1051	-0.203	-0.255	-0.119	-0.197	0.012	0.001	-0.201	-0.271	-0.123
							-0.662 **	-0.347	-0.912 ***	-0.889 ***	-0.184	-0.671 **	-0.644 **	0.546 *	-0.842 ***
Chl a								0.709 ***	0.789 ***	0.863 ***	0.631 **	0.766 ***	0.983 ***	0.055	0.564 *
Chl b									0.461	0.549 *	0.209	0.286	0.790 ***	0.413	0.001
Fv/Fm										0.979 ***	0.419	0.817 ***	0.778 ***	-0.392	0.816 ***
Fv/Fo											0.500 *	0.829 ***	0.849 ***	-0.258	0.774 ***
A_N												0.6657 **	0.586 *	0.202	0.323
Fe leaf													0.722 ***	-0.282	0.741 ***
Fe root														0.102	0.481 *
FC-R															-0.621 **
H^+ efflux															

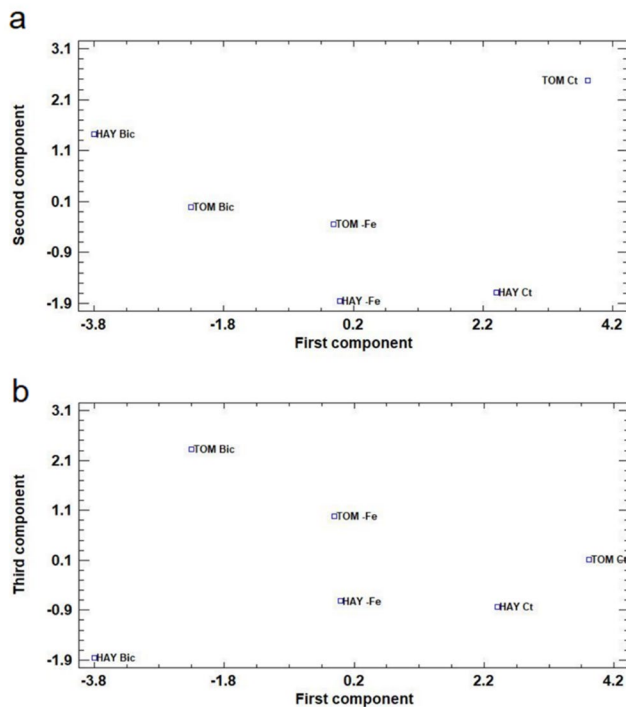


Fig. 8 Similarities among the kiwifruit plants evaluated according to physiological responses in plants when grown under the Fe-deficiency conditions, represented in: the two first components (a) (first component, x-axis; second component, y-axis) of the principal components analysis (53.35% and 19.43% of total variation, respectively); the first and third components (b) (first component, x-axis; third component, y-axis) of the principal components analysis (53.35% and 14.77% of total variation, respectively). Ct: control (20 μ M o, o-FeEDDHA); -Fe: 0 μ M Fe; Bic: 20 μ M o, o-FeEDDHA + 10 mM NaHCO_3

right of the plot, while the most yellow-colored and those with the lowest Fe contents to the left of the plot. The highest correlations in PC2 were recorded for leaf DW, the chlorophyll b concentration and FC-R activity, while negative correlations were found for fibrous root DW and H^+ efflux. The plants with the biggest leaf number, the highest chlorophyll b level and the best FC-R capacity were placed in the upper part of the plot, with the plants with the fewest fibrous roots and the lowest H^+ extrusion capacity in a lower position. So in the projection (Fig. 8A), Hayward Ct and Hayward -Fe were located close in the low plot zone, while Hayward Bic was farther away and further up from them to the left of the plot. The Tomuri -Fe and Tomuri Bic plants were located quite closely in the center of the plot, while Tomuri Ct was placed alone in its upper-right zone. In general, the -Fe plants of both cultivars were placed together in the center of the plot.

PC3 showed positive correlations with leaf number and root DW (both coarse and fibrous types), while a negative correlation was established with leaf DW. So the plants placed further up in the plot presented a big root system and a large leaf number, but with small-sized leaves. The plants

(Fig. 8B) from the three Hayward treatments were located in the lower plot zone, while the Tomuri plants were located from the middle to the top of the plot. A clear difference appeared between the Bic-treated plants because the two genotypes were located in the opposite plot zone.

4 Discussion

4.1 Effect of Fe deficient treatments on plant development

In both kiwifruit cultivars, the visible Fe deficiency symptom appeared mainly as interveinal yellowing on leaves for both no Fe in the nutrient solution and induction by bicarbonate addition (Fig. 1). Some growth parameters, such as smaller leaf area, leaf dry weight and smaller leaf number, showed that vegetative development also decreased compared to the control. However, the root system did not show any growth reduction at the end of the experiment (Fig. 1; Table 1), which suggests that the 45-day-treatment was insufficient for comprising the growth of this organ despite the low Fe concentration. Given this effect, the root/shoot ratio lowered in the Tomuri-Fe, Hayward Bic and Tomuri Bic plants (Fig. 2), which evidences Fe-chlorosis stress in both cultivars. It is not surprising that the aerial part is the first plant fraction to show symptoms because of the key role played by Fe in photosynthetic pigments and it acting as a co-factor of important processes in leaves (Abadía 1992). Similar differences between shoot and root growth sensitivity to bicarbonate have been described for pea (White and Robson 1990), (De la Guardia and Alcántara 2002), peach (Gharsalli and Hajji 2002), citrus (Martínez-Cuenca, et al. 2013) and *Arabidopsis thaliana* (Msilini et al. 2009) because Bic ion impairs Fe uptake and transport to the aerial part (Abadía et al. 1999; Pestana et al. 2005; Molassiotis et al. 2006; Jelali et al. 2011). In *A. chinensis* var. *chinensis* “Qihong”, a leaf number and leaf DW also reduced 40 days after treatment with Bic, but no statistical differences were obtained for the other parameters (i.e. stem length, stem DW, root DW) (Ksouri et al. 2007). Other authors did not find a significant effect on the percentage of stem diameter changes in six kiwifruit cultivars compared to the genotype type at different bicarbonate levels and with their interaction (Alizadeh et al. 2022). Our results agree with other studies in plants like lupin, pea, olive, peach, *M. ciliaris* and citrus (Martínez-Cuenca et al. 2013), M’Sehli et al. 2008 and references therein].

4.2 Effect on photosynthetic pigments

The yellowing symptoms in the treated plants corresponded to the lowering Chl concentration in leaves. This fact was especially notable in the Tomuri plants, which showed a marked reduction (between 68.9% and 78.9%) in both pigment types (Chl a and Chl b). The difference in the Chl b concentration between both genotypes, in addition to leaf number, was likely the key for the location of the Tomuri Ct and Hayward Ct plants on the opposite PCA plot zones (Fig. 8A). The reason for this may be related to not only Fe nutrition, but also leaf biomass and the effect of shading from leaves themselves on the pigment concentration in this cultivar. This agrees with the increment in the concentration of chlorophylls in kiwifruit, especially in chl b, under the shading conditions reported by Chartzoulakis et al. (1993). The biggest leaf biomass in the Tomuri Ct plants related to Hayward Ct (146.7% and 49.0% higher in number and total DW, respectively, Table 1) suggests a bigger shaded aerial part fraction. With the -Fe treatment, Tomuri reduced both traits (25.7% and 30.6%, respectively). Consequently, the percentage of shaded biomass lowered in Tomuri-Fe and both pigments decreased (3.2-fold and 4.3-fold in Chl a and Chl b, respectively). Although we observed yellowing symptoms in the Hayward -Fe leaves, and as no statistical differences appeared between the -Fe and Ct leaves in number or DW, Chl a decreased by 2.1-fold, while Chl b did not change.

With the presence of Bic treatment, the drop in the leaf Chl concentration in our study was as pronounced as those exhibited by the plants growing without Fe (Pestana et al. 2001). These results are consistent with other works carried out in six kiwifruit genotypes (Alizadeh et al. 2022) and in *A. chinensis* var. *chinensis* “Qihong” (Wang et al. 2019), where the total leaf chlorophyll and carotenoid content significantly declined after 20 days in the presence of bicarbonate ion content in irrigation water. A similar pattern has been observed in previous observations from field- and sand-grown kiwifruit plants (Wang et al. 2019; Liu et al. 2002; Song et al. 2003; Tran et al. 2012). Fe chelate fertilizer application improved the concentration of some pigments (lutein, β -carotene and Chl b) in the leaves of the highly calcareous sensitive *Actinidia chinensis* var. *chinensis* ‘Hort16A’ cultivar, but not Chl a (Thorp et al. 2021). In other plants, the Chl concentration reduction due to the -Fe and Bic treatments has also been observed for peach (Molassiotis et al. 2006), grapevine (Ksouri et al. 2007), *A. thaliana* (Msilini et al. 2009), pea (Jelali et al. 2011) and citrus (Martínez–Cuenca et al. 2013, 2016). The effect of treatments that enhanced Fe deficiency in Chl content has been ascribed to the role that Fe plays toward the formation of some Chl biosynthesis precursors (Marschner 1995).

4.3 Effect on fluorescence and gas exchange parameters

In relation to the low level of photosynthetic pigments, a decline in not only the maximum quantum yield of PS II (F_v/F_m), but also in the F_v/F_o ratio between variable and initial fluorescence, happened from the first time points of the experiment (15 days) under both the Fe-deficient conditions, but was slightly more marked in the Bic treatment, as reflected by the correlation analysis (Table 2). This result is in accordance with other works carried out in -Fe leaves of other fruit crops like peach and citrus (Martínez–Cuenca et al. 2016; Pestana et al. 2005; Nedunchezian et al. 1997), and in Bic-treated peach and citrus leaves (Martínez–Cuenca et al. 2013; Molassiotis et al. 2006). In grapevine, the decline in F_v/F_m was due to the decreasing chlorophyll concentration recorded in all the CaCO_3 treatments, albeit with differences between wild grapevine plants and the 41B lime-tolerant rootstock (Cambrollé et al. 2015).

The photosynthetic rate (A_N) of kiwifruit leaves moved in the same direction as the pigment concentration at the end of the experiment, especially for Chl a (Fig. 2), because both cultivars induced a significant decrease in A_N with both treatments. This effect was observed at 30 and 45 days independently of cultivar, and in Hayward Bic at 15 days after treatment began. A deleterious effect of Fe deprivation and the bicarbonate effect on the photosynthetic rate have also been reported in peach and citrus rootstocks (Martínez–Cuenca et al. 2013; Molassiotis et al. 2006). A lowering A_N value is a typical response associated with this abiotic stress and is a differential screening trait when comparing tolerance between cultivars or rootstocks of the same species. As a consequence of the drop in A_N , fewer photoassimilates are produced by plants, which implies a reduction in plant growth and yield.

When analyzing some ratios related to A_N at the end of the experiment, instantaneous carboxylation efficiency (A_N/C_i) decreased in both cultivars and treatments, which indicates metabolic limitations of photosynthesis due to Fe deficiency (Connolly et al. 2002; Vert et al. 2002). Water use efficiency (A_N/E) remained unaltered in the Tomuri cultivar, which suggests that the drop in A_N is not related to water restrictions or stress in these plants, unlike what happens when studying water-related stresses like water deficit or salt toxicity (Martínez–Cuenca et al. 2020).

In relation to plant development, the drop in A_N induced a negative effect on some vegetative parameters, like the smaller leaf number and surface, and the reduction in both leaf and stem dry weight, in the -Fe and Bic plants *versus* the control. Compared to other studies, the Hayward plants grown under the -Fe conditions showed reduced plant growth (root and shoot biomass), especially in the second

fraction (Vizzotto et al. 1997). This reduction in some growing, fruiting and fruit quality parameters in ‘Qinmei’ kiwifruit tree submitted to the -Fe treatment has been related to the A_N parameter and reductions by 62.0%–83.6% in the amount of chloroplast (Chunchao et al. 2005). Moreover, some physiological parameters, as Chl a and fluorescence, correlated negatively with the root/shoot ratio (Table 2) with the highest value for the Bic plants in the left PCA plot zone (Fig. 8A). So this parameter behaves as a good indicator of Fe chlorosis in kiwifruit, as previously stated in grapevine, where the root/shoot ratio was concluded to be an even more appropriate tool for rootstock lime-tolerance screening instead of FC-R activity or Fe concentration (Assimakopoulou et al. 2016).

4.4 Effect on reduction activity of the root system

It is assumed that the FC-R enzyme is activated by Fe deficiency (Robinson et al. 1999; Connolly et al. 2002; Vert et al. 2002; Waters et al. 2002; Li et al. 2004), and its induction is a key physiological response when screening for materials that are tolerant to iron chlorosis in fruit crops, and not only under -Fe conditions (Martínez-Cuenca et al. 2016), but also when grown in the presence of Bic (Martínez-Cuenca et al. 2013). However, we did not find this response with the -Fe treatment, which was statistically higher than Ct in the Hayward Bic roots. For this reason, the Hayward Bic plants were located further up than the Tomuri Bic plants in the PCA analysis (Fig. 8A). The bibliography about FC-R activity in *Actinidia* roots is also controversial. Some studies have obtained lesser FC-R induction in the Hayward cultivar in response to 0 μM Fe than in tolerant kiwifruit genotype D1. Two studies conducted in Hayward plants (Vizzotto et al. 1997), and in Hayward and two Italian cultivars (D1 and line 2084, classified as resistant and moderate-resistant to lime-induced conditions, respectively, Vizzotto et al. 1999), have obtained a good FC-R induction level, but the methodology differed from our study in plant growth conditions and as regards the method of measuring FC-R. More in line with our results was lack of activation of the *FRO2* gene, which controls FC-R activity in roots, in *A. chinensis* var. *deliciosa*, *A. macrosperma* and *A. polygama*, but was up-regulated in *A. chinensis* var. *chinensis* and *A. valvata* (Chen et al. 2023). The bicarbonate treatment resulted in considerable FC-R activity in the Hayward roots, but not in the Tomuri plants, and was likely mediated by the Fe concentration in both root and leaves because the Fe levels recorded for the Hayward Bic plants were statistically lower than for Hayward Ct and Hayward -Fe. The Fe concentration in the Tomuri Bic roots and leaves was no different from the Tomuri -Fe organs. We also found a moderate negative correlation between leaf DW and the

leaf Fe concentration, and a positive one with FC-R activity (Table 2), which had a dilution effect of leaf biomass and Fe-deprived conditions on leaves (sink organs) by activating the Fe reduction response in the root system. This idea is also supported by Tomuri behavior under the -Fe conditions, which lowered the Fe concentration in the root system to counteract the high Fe demand in the aerial part (62% bigger biomass than Hayward) because it maintains acidification capacity at the same level as Tomuri Ct and higher reduction activity was recorded than Hayward -Fe.

The effect of Bic on FC-R activity covers a wide range of possibilities, but no common pattern is clear in the bibliography. Like the Hayward Bic plants, some studies in citrus have observed that adding bicarbonate to a complete nutrient solution results in increased root-reducing capacity *versus* adequate Fe supply (Chen et al. 2023). A similar response has been observed in quince (Cinnelli 1995), pear (Donnini et al. 2009), peanut (Zuo et al. 2007), grapevine (Ksouri et al. 2007), *Arabidopsis* (Msilini et al. 2009) and pea (Jelali et al. 2011). *FRO2* activation has been noted in the roots of four kiwifruit genotypes (including *A. chinensis* var. *deliciosa*) grown under Bic conditions, but not in the *A. polygama* species (Chen et al. 2023), which is more in line with Tomuri Bic plant behavior. However, both responses were observed during a time-course experiment in *A. chinensis* var. *chinensis* ‘Qihong’ because FC-R activity was first induced by the Bic treatment at early time points (10 days after treatment began). Yet the tendency changed from halfway through the experiment and was lower than Ct at day 40. With 10 mM HCO_3^- , the *FRO2* gene was also activated in the sensitive rootstock *A. chinensis* var. *chinensis* ‘Qihong’, but not in the tolerant *A. chinensis* var. *deliciosa* ‘Nongdayuxiang’, nor in either the gene expression or enzymatic activity (Chen et al. 2023). Similarly, some reports in citrus and peach have also concluded that a high bicarbonate concentration in the nutrient solution may not increase FC-R activity (Chouliaras et al. 2004a; Molassiotis et al. 2006). However, the down-regulation of the enzyme in the presence of Bic has been observed in cucumber, pea, tomato and *Arabidopsis* (Romera et al. 1997; Lucena et al. 2007).

4.5 Effect on rhizosphere acidification

The Fe-deficient conditions also induced poor H^+ release in roots, especially with the Bic treatment. This result is supported by the correlation analysis (Table 2) because H^+ -extrusion was positively related to the Fe concentration in plants. The inhibition of the rhizosphere acidification process by this ion occurred in *A. chinensis* var. *deliciosa* ‘Nongdayuxiang’ (Chen et al. 2023) and other bicarbonate-treated crops, such as peach (Molassiotis et al. 2006), citrus (Martínez-Cuenca et al. 2013), *A. thaliana* (Msilini et al.

2009) and pea (Jelali et al. 2011). The sensitive *A. chinensis* var. *chinensis* ‘Qihong’, with pH increasing by 2 points, changed, as did the *HA8* gene expression with the Bic treatment (Chen et al. 2023).

This behavior is contrary to the acidification of the medium, which helps to mobilize Fe toward soluble forms, and is reported as one of the strategy I mechanism steps (Rabotti et al. 1995). The bicarbonate ions present in the root apoplast likely neutralize the protons pumped out of the cytosol and comprise the transmembrane electrochemical gradient for Fe^{2+} transport inside the cell (Zocchi and Cocucci 1990). In addition, Fe ions can be trapped in the root apoplast when bicarbonate is present because it blocks the reduction of apoplast pH and, therefore, Fe translocation to the aerial part (Kosegarten and Koyro 2001). H^+ -ATPase is the enzyme involved in H^+ extrusion, and its activity is induced by Fe deficiency to lower pH in the rhizosphere and to solubilize the ferric forms of the Fe present in soil. Its activity is regulated by two main genes, *HA1* and *HA2*, used to increase their activity when Fe-deficient conditions occur, even in the presence of Bic, as noted in pea (Jelali et al. 2011), cucumber (Santi et al. 2005), citrus (Martínez-Cuenca et al. 2013) and pear (Donnini et al. 2009). However, some works have observed that *HA2* gene activity in cucumber plants (Lucena et al. 2007) and quince genotypes (Donnini et al. 2009) is not altered by the -Fe or the Bic treatment, whereas that of *HA1* is reduced by both treatments (Lucena et al. 2007), which is in accordance with our results about H^+ being extruded in both *Actinidia* cultivars. The H^+ -ATPase gene (*HA8*) was not induced by the Bic treatment in tolerant kiwifruit *A. chinensis* var. *deliciosa* ‘Nongdayuxiang’, while H^+ efflux and *HA8* expression were activated in the sensitive cultivar *A. chinensis* var. *chinensis* ‘Qihong’ (Chen et al. 2023). However, both responses were repressed in *A. chinensis* var. *chinensis* ‘Qihong’ from day 28 onward in another study (Wang et al. 2019). Despite being contradictory, experiments were not comparable and differed markedly in the Bic treatment. The Bic composition in the first study (Chen et al. 2023) was $4 \text{ g l}^{-1} \text{CaCO}_3 + 0.84 \text{ g l}^{-1} \text{NaHCO}_3$, which falls in line with our experimental design with 10 mM, while the Bic solution in the second study (Wang et al. 2019) was lower: $0.25 \text{ g l}^{-1} \text{CaCO}_3 + 0.42 \text{ g l}^{-1} \text{NaHCO}_3$.

5 Conclusions

Under Fe-deprived conditions, 1/Tomuri cultivar presented a more optimized system for mobilizing Fe from the root system, and for maintaining photosynthetic activity and physiological functions in the aerial part, with similar H^+ -extrusion to the Ct plants and FC-R activity was greater

than Hayward -Fe; 2/The more marked drop in the Chl concentration in the Tomuri Fe-deprived plants seemed to be a combined effect of the strong correlation with the Fe content in roots and also with a certain, albeit more attenuated, effect of a small shading biomass, while the more weakened decrease in Hayward depended only on ion nutrition; 3/ under the bicarbonate conditions, strategy I was not activated in any *Actinidia deliciosa* cultivar, probably because of roots’ capability to acidify the growth medium weakens with prolonged stress, and also because FC-R activity is very pH-dependent.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13580-024-00613-7>.

Acknowledgments and funding This research was funded by MCIN/AEI/10.13039/501100011033 and the European Union “NextGenerationEU”/PRTR with project reference TED2021-132755B-I00.

Author contributions Conceptualization, M.A.F.G. and M.R.M.C.; Funding acquisition, M.A.F.G.; Methodology, A.P.C., L.C.S. and M.R.M.C.; Project administration, M.A.F.G.; Supervision, M.A.F.G.; Writing – original draft, M.R.M.C.; Writing – review & editing, A.P.C., L.C.S. and M.A.F.G.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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