HortScience 43(2):304–309. 2008.

Tolerance Response to Iron Chlorosis of *Prunus* Selections as Rootstocks

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Additional index words. Ferric chelate reductase, hydroponics, SPAD

Abstract. **The use of rootstocks tolerant to iron deficiency represents the best alternative to prevent Fe chlorosis for peach production in calcareous soils. Early detection laboratory screening procedures allow the selection of new Fe-efficient rootstock genotypes. Seventeen *Prunus* rootstocks were tested for root ferric chelate reductase (FC-R) enzymatic activity, leaf SPAD values, and field performance. Some rootstocks were used as a reference to compare with new *Prunus* selections. Micropropagated plants were grown in hydroponic culture with half-strength Hoagland's nutrient solution containing 90 μM Fe(III)-EDTA as a control treatment. Plants were transferred to iron-free fresh solutions for 4 days and were thereafter resupplied with 180 μM Fe(III)-EDTA for 1 or 2 days. In vivo FC-R activity was measured in all treatments, i.e., control, Fe-deficient, and 180 μM Fe(III)-EDTA resupplied plants. The FC-R activity after Fe resupply was higher in Fe-efficient genotypes such as Adesoto^{PVP}, Felinem^{PVP}, GF 677, Krymsk 86™, and PAC 9921-07 than in the controls. No induction of FC-R activity was found in other genotypes such as Barrier, Cadaman™-Avimag^{PVP}, PAC 9907-23, and PAC 9908-02. An intermediate response was observed in Garnem^{PVP}, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, and PAC 9904-01. According to the induction of FC-R activity after Fe resupply, genotypes were classified as tolerant, moderately tolerant, or nontolerant to iron-induced chlorosis. These results were compared with SPAD values of plants grown under controlled conditions and in the nursery. Rootstocks that show high induction of FC-R activity also showed high or very high SPAD values in the field.**

Iron is an essential micronutrient for plant growth and development because of its importance in numerous cellular functions. Low iron bioavailability is mainly the result of its insolubility at higher pH values, especially in calcareous soils, where roots are unable to acquire Fe (Hell and Stephan, 2003).

Lime-induced Fe deficiency has a strong effect in the production of several fruit crops of high economic importance grown in calcareous soils. Iron chlorosis is a common problem in peach, pear, quince, kiwi, and citrus (Tagliavini and Rombolà, 2001), is known to reduce fruit yield and quality, and to affect tree growth (Almaliotis et al., 1995). In addition, it delays fruit ripening (Álvarez-Fernández et al., 2003) and increases orchard management costs (Abadía et al., 2004; Sanz et al., 1992).

The use of Fe chlorosis-tolerant genotypes as rootstocks represents a reliable solution to prevent iron chlorosis (Socias i Company et al., 1995; Tagliavini and Rombolà, 2001). The GF 677 rootstock has a high tolerance to iron chlorosis (Cinelli et al., 2004; Giorgi et al., 2005; Socias i Company et al., 1995). Despite it being widely present throughout Europe, GF 677 is becoming obsolete because of its susceptibility to *Agrobacterium tumefaciens* and root-knot nematodes (Fernández et al., 1994), and excess vigor (Tagliavini and Rombolà, 2001), nonsuitable in the present production systems (DeJong et al., 1999). Subsequently, it is necessary to find and test new tolerant genotypes to lime-induced chlorosis, which must fulfill other agronomic requirements such as compatibility with the grafted cultivar and tolerance or resistance to soil-borne pests and diseases (Reighard et al., 2006) to use them as commercial rootstocks. Traditional selection procedures used to detect tolerance to iron chlorosis are based on field evaluation (Socias i Company et al., 1995). However, this practice requires long evaluation periods, is time consuming, and is very expensive. Therefore, rootstock breeding programs should benefit from new evaluation methods that would enable early detection of iron chlorosis. Laboratory methods based on plant physiological responses to chlorosis can be used for early selection of tolerant plant genotypes (Jolley et al., 1996) that must be confirmed later in field conditions. Several studies to select new genotypes tolerant to iron chlorosis in woody plants have been based on the root capacity to reduce Fe-chelates (Dell'Orto et al., 2000; Gogorcena et al., 2000, 2004; Marino et al., 2000; Romera et al., 1991b), the chlorophyll content of leaves (Cinelli and Loreti, 2004; De la Guardia and Alcántara, 2002), or the root organic acid content (Jiménez et al., 2007; Ollat et al., 2003).

Two mechanisms have been described to acquire Fe from the growth medium under iron deficiency: one in dicots and nongraminaceous monocots called Strategy I and another one in graminaceous monocots called Strategy II. In general, Strategy I plants show morphological and cytological changes in the roots, e.g., swelling of the root tips, and formation of root hairs and transfer cells in the root epidermis. Possible physiological changes of this mechanism include acidification of the rhizosphere, exudation of reducing and chelating substances, and increase in ferric chelate reductase (FC-R) activity (reviewed in Schmidt, 1999), which reduces Fe^{3+} and enables uptake via an Fe transporter (Briat and Lobréaux, 1997). The FC-R increases the Fe^{2+} concentration at the cell surface for uptake by the roots (Moog and Brüggemann, 1994). Unlike graminaceous monocots, reduction of Fe^{3+} to Fe^{2+} is obligatory for absorption in Strategy I plants (Chaney et al., 1972; Yi and Guerinot, 1996). In iron-efficient plants, these induced iron deficiency responses are obviously enhanced under iron shortage (Bienfait et al., 1983; Fox et al., 1996).

The aim of this study was to evaluate the Fe-deficiency induction of in vivo root FC-Ractivity in 17 commercial and experimental *Prunus* rootstocks. The results obtained with the screening protocol were compared with leaf SPAD readings obtained under field conditions that induced iron chlorosis.

Received for publication 12 July 2007. Accepted for publication 15 Oct. 2007.
Financial support was provided by Interministerial Commission of Science and Technology (nos. AGL2002-04219, AGL2003-01999, and AGL2005-05533), PETRI (no. PTR1995-0580), and DGA (nos. A28 and A44), and was cofunded by the European Regional Development Fund. S. Jiménez was supported by an I3P fellowship from CSIC/FSE (Spanish Council for Scientific Research/European Social Fund).
We gratefully acknowledge M. Cunill, P. Sánchez, and A. Santos for plant material management, and T. Zaharieva and G. Reighard for critical reading of this manuscript.
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Materials and Methods

Plant material and growth conditions. The plant material used in this investigation belonged to the breeding programs of Agromillora Catalana S.A. (Barcelona, Spain), the Estación Experimental de Aula Dei (Zaragoza, Spain), and others.

Micropropagated *Prunus* rootstocks (Adesoto^{PVP}, Barrier, Cadaman™-Avimag^{PVP}, Felinem^{PVP}, Garnem^{PVP}, GF 677, Gisela 5^{PVP}, Krymsk 1^{PVP}, Krymsk 86™, Torinel™-Avifel^{PVP}, VSL-2™, PAC 9904-01, PAC 9907-02, PAC 9907-23, PAC 9908-02, PAC 9921-07, and PAC 0006-05) were obtained from Agromillora Catalana S.A. (Barcelona, Spain). The origin and species composition of these rootstocks are listed in Table 1. Plants were grown for 2 weeks in 300-cm^3 pots containing a peat substrate. Thirty-four plants of each genotype were then transferred to 10-L plastic containers filled with half-strength Hoagland's nutrient solution. The nutrient solution was composed of 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 46.2 μM H_3BO_3, 9.2 μM $MnCl_2$, 0.38 μM $CuSO_4$, 2.4 μM $ZnSO_4$, 0.12 μM Na_2MoO_4 (pH 6.0), and 90 μM Fe(III)-EDTA. Plants were grown in a continuously aerated nutrient solution in a growth chamber under controlled environmental conditions, with a 16-h photoperiod (220 μmol m^{-2} s^{-1}) at 23 °C and 8 h of darkness at 20 °C with the relative humidity maintained at 70% to 75%. The nutrient solution was changed every 4 days, and the pH was checked and readjusted to 6.0 if necessary every 2 days, unless otherwise stated.

After stems and roots were about 15 and 10 cm long, respectively, plants were submitted to two treatments: 18 plants were transferred to iron-free solution (–Fe) and six were kept in solution containing 90 μM Fe(III)-EDTA (+Fe). After 4 days, 12 (–Fe) plants were resupplied with 180 μM Fe(III)-EDTA for 1 (180-1d) or 2 (180-2d) days. There were four different nutrient solution treatments for each genotype, namely: deficient (–Fe) (5 days without iron), control (+Fe), and resupply (180-1d) and (180-2d).

On the other hand, 20 plants of each genotype were planted in the nursery of the Estación Experimental de Aula Dei. Plants were grown on calcareous soil having 29% to 31% total calcium carbonate, 8.1% to 9.9% active lime, pH 9.0, and a silt-loam texture.

Measurement of in vivo root reduction with whole plants. In vivo root FC-R activity of intact plants was carried out in the growth chamber. Plant roots were rinsed in deionized water, and individual plants were transferred to 50-mL black plastic beakers covered with tape to exclude light and containing 49 mL of 300 μM bathophenanthrolinedisulfonic acid (BPDS; ACROS Organics, Geel, Belgium) and 10 mM MES, pH 6.0, continuously aerated. One milliliter of Fe(III)-EDTA (Sigma, St. Louis) was added to a final concentration of 500 μM. After one hour, 1-mL aliquots were taken and centrifuged to exclude any solid particles. Reduction rates of Fe(III) were estimated spectrophotometrically from the formation of the Fe(II)-$BPDS_3$ colored complex at 535 nm and an extinction coefficient of 22.14 $mM^{-1}·cm^{-1}$ (Bienfait et al., 1983; Chaney et al., 1972; Cinelli et al., 1995; Gogorcena et al., 2000) after subtraction of blanks. Blank solutions were estimated without plants. At least five plants were used as replicates for each treatment.

SPAD measurements. The iron chlorosis level was assessed with a SPAD-502 Chlorophyll Meter [Minolta Camera Co. (no longer in business as a camera company)] in ungrafted plants grown in the climatic chamber and in the field. The second, fully expanded leaf was used for the SPAD measurement in plants grown in the chamber. Values of the 2 d of resupply were averaged (180).

In 2003, SPAD values of plants established in the nursery of the Estación Experimental de Aula Dei were obtained in the months of July and August from 1-year-old ungrafted plants. Ten young fully expanded leaves from different areas of each plant were measured to obtain an average leaf SPAD value. At least five replicates were taken per rootstock.

Leaf mineral analysis. Leaf samples were collected from plants after measuring the FC-R activity of the different treatments. The leaves were subjected to three consecutive washings (HCl diluted, soapy water, and deionized water) for removing elements adhered on their surface and afterward were dried in a forced air oven at 60 °C and ground up for mineral analysis.

The mineral elements of the dried samples were measured using the methods of C.I.I. (1969) and C.I.I. et al. (1975). Nitrogen was determined by Kjeldahl analysis; P was analyzed by ultraviolet spectrophotometry (8452 A; Hewlett-Packard, Palo Alto, CA); K by atomic emission spectroscopy, and Ca, Mg, Fe, Mn, Cu, Na, and Zn by atomic absorption spectroscopy (1100; Perkin-Elmer, Norwalk, CT).

Data analysis. Data were evaluated by analysis of variance with SPSS 13.0.1 (SPSS, Chicago). When the F test was significant, means were separated by Duncan's multiple range test ($P \leq 0.05$). Regression analysis was done between the FC-R activity ratio and the SPAD values of resupplied treatments under controlled conditions. The atypical data of the Krymsk 86™ mean ratio was not included in the Pearson's correlation analysis.

Table 1. Rootstocks species and origin.

Rootstock[z]	Species	Origin[y]
Adesoto^{PVP}	*P. insititia*	CSIC, Spain
Barrier	*P. persica* × *P. davidiana*	CNR, Italy
Cadaman™-Avimag^{PVP}	*P. persica* × *P. davidiana*	INRA, France and Hungary
Felinem^{PVP}	*P. dulcis* × *P. persica*	CITA, Spain
Garnem^{PVP}	*P. dulcis* × *P. persica*	CITA, Spain
GF 677	*P. dulcis* × *P. persica*	INRA, France
Gisela 5^{PVP}	*P. canescens* × *P. cerasus*	UG, Germany
Krymsk 1^{PVP} (VVA-1)	*P. cerasifera* × *P. tomentosa*	KEBS, Russia
Krymsk 86™	*P. cerasifera* × *P. persica*	KEBS, Russia
Torinel™-Avifel^{PVP}	*P. domestica* × *P. spinosa*	INRA, France
VSL-2™	*P. fruticosa* × *P. lannesiana*	KEBS, Russia
PAC 9904-01	(*P. davidiana* × *P. persica*) × (*P. dulcis* × *P. persica*)	AC, Spain
PAC 9907-02	*P. dulcis* × (*P. persica* × *P. persica*)	AC, Spain
PAC 9907-23	(*P. dulcis* × *P. persica*) × *P. persica*	AC, Spain
PAC 9908-02	(*P. dulcis* × *P. persica*) × *P. persica*	AC, Spain
PAC 9921-07	(*P. besseyi* × *P. salicina*) × *P. armeniaca*	AC, Spain
PAC 0006-05	(*P. dulcis* × *P. persica*) × *P. persica*	AC, Spain

[z]Next the Rootstock, ^{PVP}Plant Variety Protection by Community Plant Variety Office in the European Union (2006), ™Trademark.

[y]AC = Agromillora Catalana, S.A. private nursery, Spain; CITA = Centro de Investigación y Tecnología Agroalimentaria de Aragón; CNR = Centro Nacionale della Recerca; CSIC = Consejo Superior de Investigaciones Científicas; INRA = Institut National de la Recherche Agronomique; UG = University of Giessen; KEBS = Krymsk Experiment Breeding Station.

Results and Discussion

FC-R activity. Seventeen commercial and experimental *Prunus* rootstocks were tested for their tolerance to Fe deficiency. For a better evaluation of the results, GF 677 rootstock was used as a reference rootstock. Increases in FC-R activity were not found in Fe-deficient *Prunus* plants compared with the control treatment (Fig. 1), but the complete lack and then the later addition of Fe may trigger increases in FC-R activity (Fig. 1; Gogorcena et al., 2004). The FC-R activity in control and resupplied plants varied widely among the evaluated genotypes (1–13 nmol Fe^{2+} $min^{-1}·g^{-1}$ fresh weight and 1–42 nmol Fe^{2+} $min^{-1}·g^{-1}$ fresh weight, respectively). To determine the induction capacity of the FC-R, the ratio between FC-R of plants resupplied with 180 μM Fe(III)-EDTA during 1 or 2 d and the activity of control plants was calculated. According to maximum activity ratios, three groups of responses were established (Table 2): higher than 3.9, between 3.0 and 1.9, and the third with no induction or induction lower than 1.8.

Some of the rootstocks, such as Adesoto^{PVP}, Felinem^{PVP}, Krymsk 86™, and PAC 9921-07, showed higher FC-R activity after 1 or 2 d of Fe resupply than did GF 677 (Fig. 1A). Within this group, PAC 9921-07 rootstock showed the highest FC-R activity in the control and deficient treatments (7.6 and

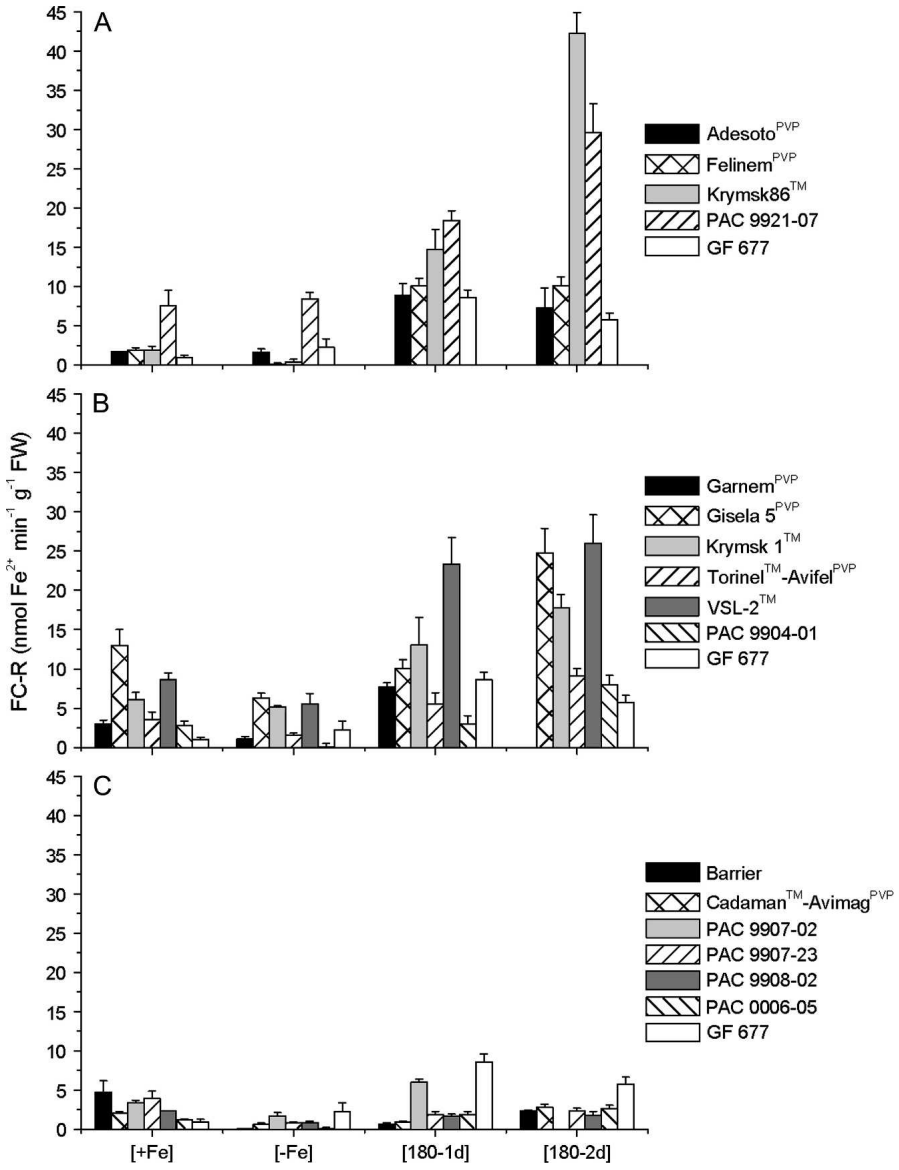

Fig. 1. Root FC-R activity (nmol Fe^{2+} $min^{-1}·g^{-1}$ fresh weight) of Adesoto^{PVP}, Felinem^{PVP}, Krymsk 86™, PAC 9921-07, and GF 677 (**A**); Garnem^{PVP}, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, PAC 9904-01, and GF 677 (**B**); and Barrier, Cadaman™-Avimag^{PVP}, PAC 9907-02, PAC 9907-23, PAC 9908-02, PAC 0006-05, and GF 677 (**C**) plants submitted to different treatments: control with 90 μM FeIII-EDTA (+Fe), 5 days Fe deficient (–Fe), and 4 days deficient plants resupplied with 180 μM FeIII-EDTA during 1 and 2 d, (180-1d) and (180-2d), respectively. The chlorosis-tolerant genotype GF 677 is included as reference. Vertical bars indicate SE. At least five plants were used for treatment and rootstock.

8.5 nmol Fe^{2+} $min^{-1}·g^{-1}$ fresh weight, respectively). For all genotypes in Fig. 1A (Adesoto^{PVP}, Felinem^{PVP}, Krymsk 86™, PAC 9921-07, and GF 677), FC-R activity increased more than 3.9-fold after Fe resupply compared with the deficient treatment (Table 2). Likewise, Krymsk 86™ showed the highest increase in FC-R activity 2 days after iron addition (180-2d) (22.1-fold). Some of these genotypes were already assessed for Fe deficiency tolerance in field and controlled conditions. The plum rootstock Adesoto^{PVP} was classified as tolerant to Fe chlorosis in the field (Moreno et al., 1995) and was also found to have a high FC-R activity rate (Gogorcena et al., 2004; Romera et al., 1991a; Santos, 2002). The peach-almond hybrid Felinem^{PVP} was evaluated under field conditions with a high lime content and showed a similar response to GF 677 (Felipe et al., 1997), probably because of the influence of its chlorosis-tolerant almond pedigree.

The FC-R activity of resupply treatments of Garnem^{PVP}, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, and PAC 9904-01 rootstocks was similar or greater than the GF 677 values (Fig. 1B), but the control FC-R activity was also greater, especially for Gisela 5^{PVP}, Krymsk 1^{PVP}, and VSL-2™ (13.0, 6.1 and 8.6 nmol Fe^{2+} $min^{-1}·g^{-1}$ fresh weight, respectively). The rootstocks Garnem^{PVP}, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, and PAC 9904-01 showed moderate increases of FC-R activity, with maximum values between 1.9 and 3.0 (Table 2). Thus, the genotypes of this group showed an intermediate response.

Table 2. Induction of root FC-R activity 1 and 2 d after Fe resupply. Ratios were calculated as activity in plants resupplied with 180 μM Fe(III)-EDTA during 1 or 2 days per activity of control treatment with 90 μM Fe(III)-EDTA [FC-R (180-1d) per FC-R (+Fe) and FC-R (180-2d) per FC-R (+Fe), respectively].

Rootstock	FC-R (180-1d) per FC-R (+Fe)[z]	FC-R (180-2d) per FC-R (+Fe)[z, y]
Adesoto^{PVP}	5.3	4.4
Felinem^{PVP}	5.3	5.3
Krymsk 86™	7.7	22.1
PAC 9921-07	2.4	3.9
GF 677	8.9	5.9
Garnem^{PVP}	2.6	nd
Gisela 5^{PVP}	ni	1.9
Krymsk 1^{PVP}	2.1	2.9
Torinel™-Avifel^{PVP}	1.6	2.6
VSL-2™	2.7	3.0
PAC 9904-01	1.1	2.9
PAC 0006-05	1.6	2.2
Barrier	ni	ni
Cadaman™-Avimag^{PVP}	ni	1.4
PAC 9907-02	1.8	nd
PAC 9907-23	ni	ni
PAC 9908-02	ni	ni

[z]ni: no induction.
[y]nd: not determined.

On the other hand, the FC-R activity of Fe resupply treatments of Barrier, Cadaman™-Avimag^{PVP}, PAC 9907-02, PAC 9907-23, PAC 9908-02, and PAC 0006-05 rootstocks was lower than that in GF 677 (Fig. 1C). These rootstocks showed little or no increase in FC-R activity when resupplied versus the (+Fe) treatment (Table 2). The slightly higher increase of FC-R activity of PAC 0006-05 rootstock was because of a low control FC-R activity (Fig. 1C). The results for Barrier and Cadaman™-Avimag^{PVP} agree with those obtained by Gogorcena et al. (2004) using the same technique. Evaluation of Barrier on calcareous soil showed lower tolerance to chlorosis than Adesoto^{PVP}, Felinem^{PVP}, and GF 677 (Iglesias et al., 2004). Also, a recent study by Molassiotis et al. (2006) reported a higher sensitivity to iron chlorosis of Cadaman™-Avimag^{PVP} than GF 677.

The method used in this study was based on the induction of chelate reduction capacity in Fe-deficient peach roots (Gogorcena et al., 2000). With this methodology, Fe-deficient rootstocks show a change in FC-R activity and the response can be measured (Gogorcena et al., 2004). Other investigations on dry bean in hydroponics showed that root iron reducing capacities were highly negatively correlated with visual chlorosis scores from field trials (Ellsworth et al., 1997) and provide a better screening ability than H^+ ion release (Ellsworth et al., 1998). Moreover, under the growing conditions of this work, rootstocks did not induce acidification of the medium with iron deficiency. A high reduction capacity will allow the plant to increase Fe uptake under soil conditions inducing Fe deficiency, as it occurs on calcareous, high pH soils. The genotypes showing higher or moderate reduction capacities will have better adaptability to calcareous soils.

SPAD values and leaf Fe concentration. In hydroponic culture, leaves of all rootstocks had lower SPAD values in the (–Fe) than (+Fe) treatments (Table 3). After 2 days of Fe resupply, rootstocks achieved SPAD units similar to control values [±10% of (+Fe)], except for Garnem[PVP], PAC 9904-01, PAC 0006-05, Barrier, Cadaman™-Avimag[PVP], and PAC 9907-02. Results of leaf mineral content analysis after Fe resupply indicated that the rootstocks with high reduction capacity had higher Fe concentrations than control plants (Table 3). The concentration of Fe in the resupply (180) treatment was considerably higher than the control for Adesoto[PVP], Felinem[PVP], Krymsk 86™, PAC 9921-07, GF 677, Torinel™-Avifel[PVP], and VSL-2™. The SPAD values of genotypes PAC 9904-01 and PAC 0006-05 were not recovered, although the induction of the FC-R was moderate to low (Table 2). Genotypes that did not induce the FC-R activity reached a similar or lower Fe concentration to that of control plants. However, the recovery in SPAD values and Fe concentration was similar to their respective control levels for the PAC 9907-23 and PAC 9908-02 genotypes. The increase in leaf iron concentrations of Adesoto[PVP] and GF 677 and the lower leaf iron concentration of Barrier and Cadaman™-Avimag[PVP] after Fe resupply was previously reported (Gogorcena et al., 2004; Santos, 2002). The rootstocks with high reduction capacity may increase the Fe^{2+} concentration at the cell surface for root uptake (Moog and Brüggemann, 1994) and the consequent translocation to the shoot, leading to high leaf Fe concentration. On the other hand, a significant positive correlation was found between the FC-R activity ratio of resupply per control treatment and SPAD values of resupplied plants (180) ($P \leq 0.05$; Fig. 2). However, the high SPAD values for PAC 9907-23 caused a not high correlation coefficient. According to these data, it is likely that the stimulation of the FC-R activity was accompanied by decrease of symptoms of iron deficiency (higher SPAD values).

In the field, SPAD readings measured in ungrafted genotypes were very high for the genotypes with increased FC-R activities such as Adesoto[PVP], Krymsk 86™, and PAC 9921-07 (Table 4). Felinem[PVP] and GF 677 had also high SPAD values, as observed by Felipe et al. (1997). Genotypes with intermediate FC-R activity responses showed intermediate SPAD values, although rootstocks such as Torinel™-Avifel[PVP] showed high SPAD readings. This could be caused by morphological genotype differences due to thickness and leaf chlorophyll density. In addition, leaf natural color of plum is darker than *P. dulcis* × *P. persica* hybrids. Thus, variation between high and medium reduction capacities could not be fully explained. Conversely, rootstocks with a low reduction capacity showed SPAD readings in the field

Table 3. SPAD values of the second fully expanded leaf from the apex of plants grown with 90 µM Fe(III)-EDTA (+Fe), without Fe for 5 d (–Fe) and 4 d without Fe and then resupplied with 180 µM Fe(III)-EDTA for 1 (180-1d) or 2 d (180-2d) [average of two days, (180)]. The recovery of SPAD values and leaf Fe concentration in deficient plants after resupply was indicated as: = plants after iron resupply reached similar values than control (±10%); < plants after resupply showed lower values than control; > plants after resupply reached higher values than control. SPAD data are means ± SE of five replicates.

	SPAD			SPAD	Fe concentration
Rootstock	(+Fe)	(–Fe)	(180)	Recovery	Recovery[z]
Adesoto[PVP]	24.1 ± 0.7	19.4 ± 1.9	25.2 ± 0.8	=	>
Felinem[PVP]	25.2 ± 0.8	22.2 ± 0.6	24.9 ± 0.9	=	>
Krymsk 86™	20.0 ± 1.7	10.0 ± 0.1	20.5 ± 1.3	=	>
PAC 9921-07	24.8 ± 2.0	15.4 ± 1.4	23.5 ± 0.9	=	>
GF 677	27.1 ± 0.7	22.2 ± 3.5	26.1 ± 0.9	=	>
Garnem[PVP]	23.1 ± 0.9	21.2 ± 0.6	19.7 ± 1.1	<	<
Gisela 5[PVP]	19.5 ± 1.1	16.5 ± 0.8	20.4 ± 2.2	=	=
Krymsk 1[PVP]	18.0 ± 1.4	13.6 ± 8.1	16.9 ± 3.6	=	=
Torinel™-Avifel[PVP]	24.1 ± 1.1	22.5 ± 0.7	23.6 ± 0.4	=	>
VSL-2™	19.7 ± 2.3	16.3 ± 0.7	20.5 ± 1.6	=	>
PAC 9904-01	17.5 ± 1.0	11.9 ± 1.0	15.0 ± 0.6	<	nd
PAC 0006-05	17.6 ± 1.9	6.4 ± 1.2	10.1 ± 1.2	<	nd
Barrier	18.1 ± 2.9	9.2 ± 0.5	12.8 ± 1.3	<	<
Cadaman™-Avimag[PVP]	18.5 ± 1.6	13.4 ± 1.8	16.5 ± 1.2	<	<
PAC 9907-02	25.2 ± 1.7	11.8 ± 2.3	21.3 ± 2.7	<	=
PAC 9907-23	26.0 ± 0.9	22.3 ± 0.4	25.5 ± 0.8	=	=
PAC 9908-02	20.9 ± 1.2	17.0 ± 1.3	21.1 ± 0.7	=	=

[z]nd: not determined.

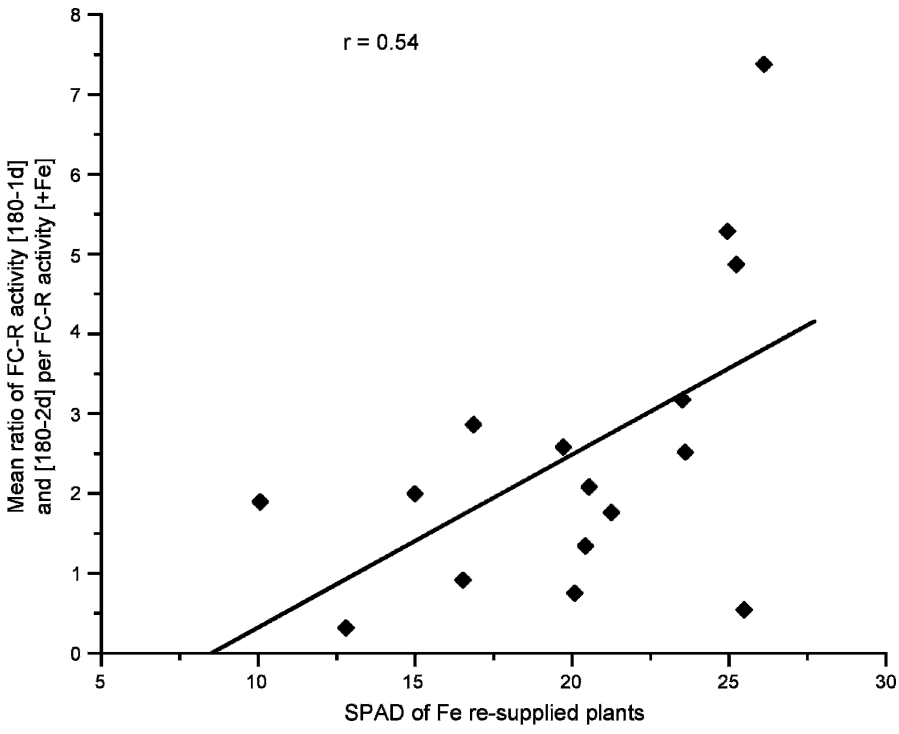

Fig. 2. Relationship between the mean ratio of FC-R activity of Adesoto[PVP], Barrier, Cadaman™-Avimag[PVP], Felinem[PVP], Garnem[PVP], GF 677, Gisela 5[PVP], Krymsk 1[PVP], Torinel™-Avifel[PVP], VSL-2™, PAC 9904-01, PAC 9907-02, PAC 9907-23, PAC 9908-02, PAC 9921-07, and PAC 0006-05 plants resupplied with 180 µM Fe(III)-EDTA during 1 (180-1d) and 2 (180-2d) days per FC-R activity of control plants (+Fe) with 90 µM Fe(III)-EDTA and SPAD values of the second fully expanded leaf from apex of deficient plants resupplied with 180 µM Fe-EDTA. Linear relation with $P \leq 0.05$ is presented.

Table 4. Leaf SPAD values of ungrafted rootstocks in nursery conditions that induce iron chlorosis. Data are means of at least five replicates.

Rootstock	SPAD[z, y]
Adesoto[PVP]	47.7 f
Felinem[PVP]	36.6 cd
Krymsk 86™	41.5 e
PAC 9921-07	41.3 e
GF 677	35.8 cd
Garnem[PVP]	35.9 cd
Gisela 5[PVP]	37.1 d
Krymsk 1[PVP]	36.3 cd
Torinel™-Avifel[PVP]	41.9 e
VSL-2™	37.2 d
PAC 9904-01	40.3 e
PAC 0006-05	nft
Barrier	28.2 a
Cadaman™-Avimag[PVP]	33.7 bc
PAC 9907-02	31.6 b
PAC 9907-23	nft
PAC 9908-02	nft

[z]Within column, means with the same letter do not differ significantly at $P \leq 0.05$ (Duncan's multiple range test).

[y]nft: not field tested. Not all genotypes evaluated under controlled conditions (hydroponic) were tested in the field.

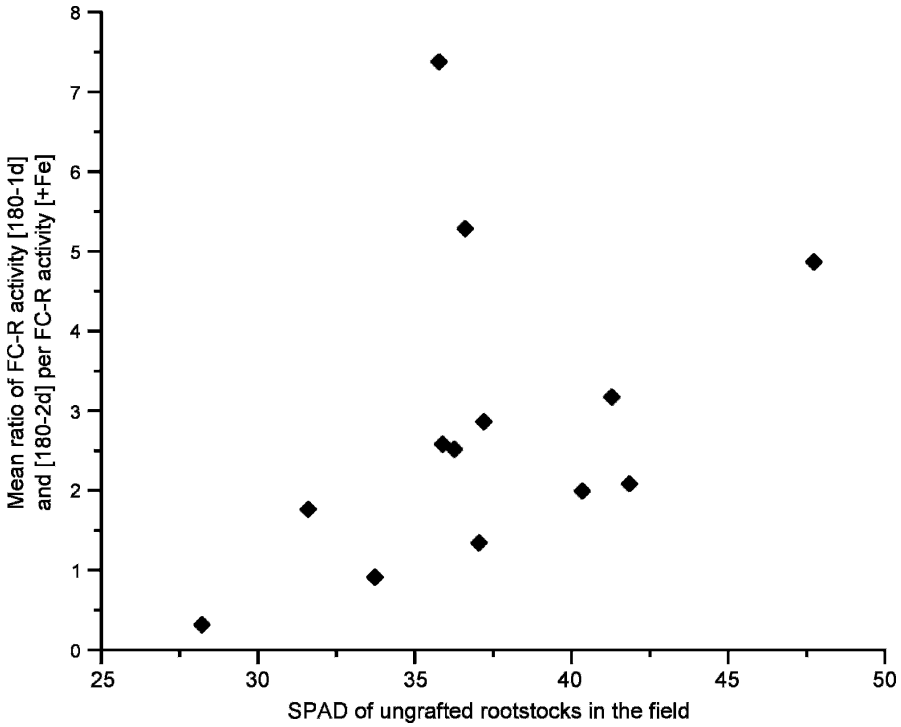

Fig. 3. Relationship between the mean ratio of FC-R activity of Adesoto^{PVP}, Barrier, Cadaman™-Avimag^{PVP}, Felinem^{PVP}, Garnem^{PVP}, GF 677, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, PAC 9904-01, PAC 9907-02, PAC 9907-23, PAC 9908-02, PAC 9921-07, and PAC 0006-05 plants resupplied with 180 μM Fe(III)-EDTA during 1 (180-1d) and 2 (180-2d) days per FC-R activity of control plants (+Fe) with 90 μM Fe(III)-EDTA and SPAD values of ungrafted rootstocks in nursery conditions that cause iron chlorosis.

below 33.7. The mean FC-R activity ratio of resupply treatments per control treatment and the field SPAD values were not significantly correlated (Fig. 3) mainly because of high FC-R activity response of Felinem^{PVP} and GF 677.

The evaluation under controlled conditions could be extrapolated to predict the response of a rootstock in the field, although bearing in mind that Fe chlorosis could be influenced by other agronomic traits such as yield, vigor, and the rootstock-scion combination effect. In the nursery, the response of grafted rootstocks in terms of leaf chlorophyll content could be different with respect to the ungrafted rootstock. Further studies with grafted plants grown under control conditions (growth chamber) should be done to elucidate the influence of the cultivar on the rootstock response to iron chlorosis, although there are scion-stock compatibility requirements that prevented grafting all rootstocks with the same cultivar.

Relative chlorosis tolerance of 17 Prunus rootstocks. With the prevailing experimental conditions, one of the main responses of the *Prunus* roots to Fe deficiency was the variation in FC-R activity. According to FC-R activity increases, preliminary rating for several rootstocks is proposed for their tolerance to iron chlorosis: tolerant (Adesoto^{PVP}, Felinem^{PVP}, Krymsk 86™, PAC 9921-07, and GF 677), moderately tolerant (Garnem^{PVP}, Gisela 5^{PVP}, Krymsk 1^{PVP}, Torinel™-Avifel^{PVP}, VSL-2™, and PAC 9904-01), and sensitive (Barrier, Cadaman™-Avimag^{PVP}, PAC 9907-02, PAC 9907-23, PAC 9908-02, and PAC 0006-05).

Many of the most tolerant accessions in the test were *P. dulcis* × *P. persica*, which is in agreement with the results obtained by Shi and Byrne (1995). However, some rootstocks (PAC 9907-02, PAC 9907-23, and PAC 9908-02) with almond parentage did not appear to be tolerant in this study, probably because they have a greater expected peach genetic background (up to 75%) than other selections. Other studies on iron chlorosis also showed a better tolerance of interspecific hybrids compared with peach seedlings (Almaliotis et al., 1995).

Tolerance to Fe-induced chlorosis is an important selection criterion for *Prunus* rootstocks in Mediterranean environmental conditions. From the practical standpoint, the increase in FC-R activity response is a useful approach of a method for early detection or screening of genotypes that are susceptible to iron chlorosis. Plant response occurs in a few weeks, allowing early selection of more tolerant genotypes in contrast to the current rootstock selection procedures that normally take several years to obtain a reliable plant response under field conditions. However, correlation of results obtained under controlled conditions with field response of grafted rootstocks need to be confirmed.

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