Journal of Agricultural Science (2014), **152**, 731–740. © Cambridge University Press 2013
doi:10.1017/S0021859613000336

CROPS AND SOILS RESEARCH PAPER

Effect of Fe deficiency on alfalfa plants grown in the presence of *Pseudomonas*

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(*Received 18 October 2012; revised 17 April 2013; accepted 8 May 2013; first published online 14 June 2013*)

SUMMARY

Alfalfa is a model plant defined as less sensitive than others to iron (Fe) deficiency. In the present work, some mechanisms induced in low Fe availability conditions were studied, including the effect of inoculation of alfalfa seeds with *Pseudomonas putida*. The effect of different Fe contents in the nutrient solution on the growth parameters was evaluated at 3 and 10 days, observing that low Fe conditions promoted biomass accumulation. Activation in the mechanisms of Fe acquisition, through acidification of the media and an increase in the ferric chelate reductase (FCR) activity, was observed in the absence of Fe at 10 days. The presence of *P. putida* KT2442 in the rhizosphere eliminated FCR activation through the excretion of siderophores. The effect of the siderophores on the modulation of FCR activity was demonstrated using a *ppsD* mutant strain, unable to segregate them, observing an activation of the activity similar to that observed in the absence of the bacteria. This, together with the demonstrated mechanisms to increase Fe availability, contributed to the conclusion that alfalfa can be used for recovery programmes of soils with low Fe availability.

INTRODUCTION

Iron (Fe) is an essential element for plants because it is required for many biological functions, including haeme and chlorophyll synthesis, and chloroplast development (Alcaraz *et al*. 1985; Miller *et al*. 1995). Iron is a co-factor for a number of metalloenzymes, cytochromes, hydroxyperoxidases, catalases and peroxidases and a constituent of ribonucleotide reductase (Hellín *et al*. 1984, 1987; Almansa *et al*. 1991). Although Fe is the fourth most abundant element in the soil, it is often unavailable to plants because it forms insoluble ferric hydroxide complexes in aerobic environments at neutral or basic pH, increasing its solubility at low pH values (Guerinot & Yi 1994). Thus, plants have developed efficient mechanisms for Fe acquisition that are directed towards increasing Fe solubility. Strategy I plants (dicots and non-grass monocots) obtain Fe from the rhizosphere through the action of membrane-bound ferric chelate reductase (FCR) and the Fe(II) is then transported into root cells by metal ion transport. Strategy II plants (all grasses) respond to Fe deficiency by releasing siderophores (small, high-affinity Fe-chelating compounds) (Römheld 1991) and inducing a specific plasma membrane Fe(III)-phytosiderophore transporter in root systems (Hell & Stephan 2003). Also, it has been reported that the microbial community, including plant growth-promoting rhizobacteria (PGPR) present in the rhizosphere, play an important role in Fe acquisition (Masalha *et al*. 2000) as well as plant Fe status, affecting the composition of siderophore-secreting microbes in the rhizosphere (Jin *et al*. 2010). In this plant-microbial interaction, vegetation plays an important role in the remediation of contaminated or deficient soils because plant roots secrete a wide spectrum of compounds, which act as growth promoters and energy substrates for microbial development in the rhizosphere and promote the proliferation of an active microbial population (Espinosa-Urgel *et al*. 2002; Houlden *et al*. 2008). In turn, organisms present in the rhizosphere have a positive impact on plants by

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producing growth-promoting compounds, increasing the availability of insoluble nutrients or suppressing the activity of pathogens (Podile & Kishore 2006). This last effect is thought to be mediated by the competitive scavenging of limiting Fe through the production of high-affinity siderophores by the growth-promoting bacteria (Ji *et al*. 2012); predominant among these are the Bacilli and Pseudomonads (Podile & Kishore 2006). *Pseudomonas* spp., an efficient root colonizer in a number of agricultural plants, responds to Fe limitation by secreting siderophores, which are synthesized by a non-ribosomal peptide synthetase coded by the *ppsD* gene in *Pseudomonas putida* (Meyer 2000; Devescovi *et al*. 2001).

Alfalfa (*Medicago sativa* L.) has been reported to grow in nutrient-deficient and contaminated soils and is able to stimulate a number of micro-organisms in the rhizosphere, principally petroleum hydrocarbons degraders (Kirk *et al*. 2005; Martí *et al*. 2009). Studies have shown that *Pseudomonas* appears to have a growth-promoting effect on alfalfa, and also mungbean (*Vigna radiata* L.) (Carrillo-Castañeda *et al*. 2002; Sharma *et al*. 2003), but the effect of the presence of *Pseudomonas* on the mobility of Fe through the activity of the FCR is unknown. Thus, the present work analysed the effect of different Fe concentrations in the nutrient solution on growth and Fe status, and some possible mechanisms involved in the response to this situation including changes in extracellular pH and in the activity of FCR in alfalfa plants. Changes induced by seed inoculation with *P. putida* (KT2442), segregating siderophores and its mutant unable to segregate them (*ppsD*), on growth and FCR activity in alfalfa plants grown in the presence and absence of Fe were also studied.

MATERIALS AND METHODS

Plant material and iron treatments

Alfalfa seeds were purchased from a local market (Ramiro Arnedo S.A.) and sterilized with 100 ml/l bleach for 10 min, placed on trays (35 × 50 × 7 cm) filled with silica sand and irrigated daily until their germination. Once germinated, seedlings were grown in control conditions of light (250 μmol/m^2/s, Philips SON-T, 400 w), 16/8 h light/dark photoperiod, 70–80% relative humidity and irrigated with distilled water for 5 days. After that, plants were irrigated with Hoagland's nutrient solution containing 5 ppm Fe (Hoagland & Arnon 1950) for 7 days prior to starting the Fe deficiency treatments (12-day old plants). Plants were then randomly divided into four groups and exposed to different Fe concentrations, i.e. 0, 0·25, 0·5 and 1·0 with the last group being used as the control treatment.

Bacterial cultures and seed inoculation

Pseudomonas strains were obtained from the *Pseudomonas* Reference Culture Collection (PRCC, Estación Experimental del Zaidín, CSIC, Spain). The rifampicin-resistant strain *P. putida* KT2442, able to produce siderophores (Franklin *et al*. 1981) was used for seed inoculation. The strain was grown at 30 °C in minimal medium (basal M9 medium supplemented with Fe-citrate, magnesium sulphate ($MgSO_4$) and trace metals) (Abril *et al*. 1989) and with benzoate (15 mM) as a carbon source. Alfalfa seeds were surface sterilized with 700 ml/l ethanol for 10 min and washed at least three times with distilled water. Seeds were inoculated by immersion for 30 min, with slow shaking, in a cell suspension of *P. putida* KT2442 or its *ppsD* mutant, which is unable to produce siderophores. One group was immersed in a sterile culture medium as the control. The seeds were placed on trays filled with silica sand to germinate and irrigated daily. After germination the seedlings were irrigated with distilled water for 5 days, then with Hoagland's solution for 7 days. The conditions of light, photoperiod and relative humidity were as described above. The control (1·0Fe) and 0Fe treatments were imposed upon plants grown from inoculated and non-inoculated seeds and morphological and biochemical measurements were performed 3 and 10 days after treatments.

Experiment 1: Growth analysis, iron status, chlorophyll content and ferric chelate reductase activity under decreasing iron content

In order to evaluate the response of alfalfa plants to decreasing Fe content, root and stem length were measured on 20 plants for each Fe level. The fresh weight of roots and aboveground biomass was measured immediately after harvesting and the dry weight was recorded after drying at 80 °C to constant weight.

Iron content was measured using oven-dried tissue, which was digested in a microwave Ethos 1 (Milestone, Italy) with nitric acid (HNO_3):hydrogen peroxide (H_2O_2) (4:1). The mineral concentration

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was determined by inductively coupled plasma spectrometry (ICP) (Iris Intrepid II; Thermo Electron Corporation, Franklin, MA, USA). Iron determination was carried out on three replicates per treatment. Under these conditions, the leaf chlorophyll content was tested with a chlorophyll meter based on measurement of leaf transmittance, a portable Konica MINOLTA soil plant analysis developer (SPAD) (Hoel & Solhaug 1998) and expressed as SPAD units. Chlorophyll measurements were carried out on ten fully expanded leaves per treatment.

The FCR activity was measured in alfalfa roots of plants after growing under the different Fe treatments (Chaney *et al*. 1972). Three plants were incubated in 50 ml Falcon tubes containing 25 ml of a solution of 0·2 mM $CaSO_4$, 5 mM 4-Morpholineethanesulfonic acid (MES), pH 5·5, 500 μM Fe^{+3}-EDTA and 0·3 mM bathophenanthrolinedisulphonate (BPDS) for 20 min. The absorbance change at 535 nm and the amount of reduced Fe were calculated by the concentration of the Fe(II)–$(BPDS)_3$ complex formed (Molar Extinction Coefficient of BPDS is 22·1/mM/cm). Results were expressed in nmol/g FW/min. Three replicates per treatment were carried out.

Experiment 2: Change of external pH under decreasing iron content

In order to examine the changes in extracellular pH, 12-day old alfalfa plants were transferred to 50 ml-Falcon tubes containing 30 ml of nutritive solution with the different Fe treatments. The pH change in the nutritive solution produced by ten plants per tube was measured with a pH electrode after 3 and 10 days of treatment. Five replicates per treatment were analysed.

Experiment 3: Growth analysis and ferric chelate reductase activity in presence of *Pseudomonas*

The aim of Expt 3 was to analyse whether the presence of *P. putida* KT2442 and its *ppsD* mutant produced any change in the plant physiology and in the FCR activity in alfalfa plants grown at 0Fe content compared with control plants. The lengths of roots and stems, as well as fresh and dry weights of roots and aboveground biomass, were measured as described above. The FCR activity was measured in control plants and inoculated plants with both *Pseudomonas* as described above (three replicates per treatment).

Statistical analysis

The experiment was conducted in a completely randomized design. The results are the mean of at least three replicates from each experiment and all experiments were repeated three times. The significance of any differences between mean values was determined by one-way analysis of variance ($P<0·05$); Duncan's multiple range tests were used to compare the means when necessary, and each different treatment was compared with the control (1·0Fe) treatment.

RESULTS

Growth parameters, Fe status, chlorophyll content and FCR activity under decreasing Fe content.

In order to explore the response of alfalfa to different Fe levels in the growing media (0·5Fe, 0·25Fe and 0Fe), the lengths of roots and stems as well as fresh and dry weights of roots and aboveground biomass were measured after 3 and 10 days, and compared with the 1·0Fe treatment. After 3 days of treatment, no significant changes were observed in any parameter (Fig. 1); however, after 10 days the root dry weight had increased significantly ($P<0·05$) from 3·4 to 5·3 mg/plant (an increase of 55·8%) in plants treated with 0·5Fe. The other Fe treatments did not affect this parameter (Fig. 1*c*). The fresh weight of aboveground biomass increased significantly ($P<0·05$) from 161·4 to 210·8 mg/plant in 0·5Fe-treated plants (an increase of 30·6%), and to 217·4 mg/plant in 0·25Fe-treated plants (an increase of 34·7%). The dry weight also increased significantly ($P<0·05$) from 20·5 to 26·0 mg/plant in 0·5Fe-treated plants (an increase of 26·8%) and to 25·3 mg/plant in 0·25Fe-treated plants (an increase of 23·4%) (Figs 1*e* and *f*).

Iron content in roots, stems and leaves showed no significant changes at 0·5 and 0·25Fe (data not shown), whereas in the 0Fe-treated plants, a significant ($P<0·05$) decrease of 9·1% was found in stems at 10 days and decreases of 17·3 and 22·4% were observed in leaves at 3 and 10 days, respectively (Table 1). Leaf chlorophyll content was not altered by 0·5 and 0·25Fe treatments at 3 or 10 days (data not shown), but decreased by 37·2% in alfalfa plants grown in total absence of Fe (0Fe) at 10 days (Table 1).

Ferric chelate reductase activity was measured at 3 and 10 days after the Fe treatments and an increase of 80% was observed in plants treated with 0Fe in the nutrient solution at 3 days (Fig. 2) when compared with the control treatment. This activity increased two-fold in the 0·25 and 0Fe treatments at 10 days.

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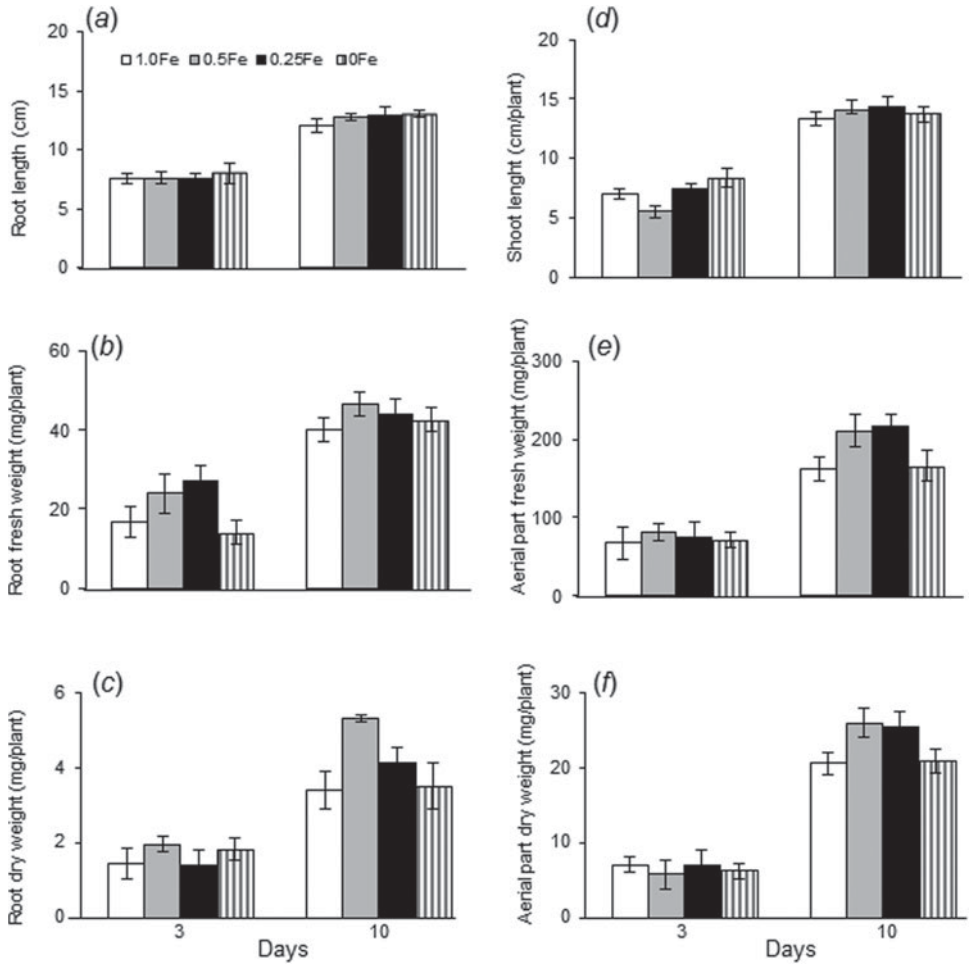

Fig. 1. Growth parameters of alfalfa plants grown for 3 and 10 days under decreasing Fe content. Values are means of three experiments ± S.E.

Changes in pH under decreasing iron content

In order to verify whether the different Fe treatments provoked acidification in the root environment, the pH of the growth solution was measured in alfalfa plants grown under hydroponic conditions after 3 and 10 days at 0·5Fe, 0·25Fe and 0Fe and compared with plants grown with 1·0Fe. No significant changes in the pH were observed in any of the treatments at 3 days (Fig. 3), but a longer exposure (10 days) to the absence of Fe provoked an acidification of the media, which was only significant ($P<0·05$) in the plants grown with 0Fe.

Effect of *Pseudomonas* inoculation on alfalfa growth and ferric chelate reductase activity

Following observation of the induction of FCR activity in plants treated with 0Fe in the nutrient solution at 3 and 10 days, an investigation was conducted into whether inoculation of seeds with *P. putida* KT2442 and its *ppsD* mutant would have any effect not only on the plant physiology but on the FCR activity induced by this condition. The effect of bacterial inoculation on the growth of alfalfa plants was analysed first and no effect was observed on any of the growth parameters measured at 3 days (data not shown). After 10 days of 1·0Fe treatment, the dry weights of roots and aboveground biomass increased by 91·7 and 47%, respectively, in the plants inoculated with *ppsD* mutant compared with the control non-inoculated plants (Table 2). At this time in the 0Fe treatment, the fresh weight of aboveground biomass increased by 46·6% in plants inoculated with the KT2442 strain compared with control non-inoculated plants.

When alfalfa plants were grown under the 1·0Fe treatment, seed inoculation with *P. putida* KT2442 or its *ppsD* mutant did not produce any change in the

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Table 1. *Fe content in root, leaf and stem and changes in leaf chlorophyll content of alfalfa plants grown in 1·0Fe (control) and 0Fe in the nutrient solution for 3 and 10 days. Values are means of three experiments* ± S.E.

	Fe			Chlorophyll
	(mg/kg)			(SPAD readings)
3 days	Root	Stem	Leaf	Leaf
1·0Fe	239 ± 30·4	100 ± 7·8	185 ± 3·8	41 ± 2·9
0Fe	255 ± 30·3	91 ± 4·9	153 ± 20·1	38 ± 2·1
10 days				
1·0Fe	199 ± 10·6	66 ± 7·3	125 ± 12·2	43 ± 1·8
0Fe	208 ± 19·4	60 ± 9·3	97 ± 3·4	27 ± 2·6

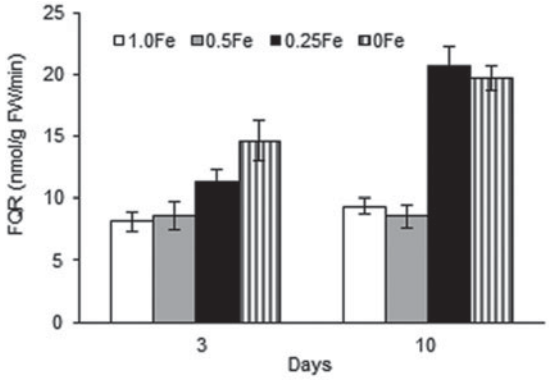

Fig. 2. Ferric chelate reductase activity of alfalfa plants grown under decreasing Fe content. Values are means of three experiments ± S.E.

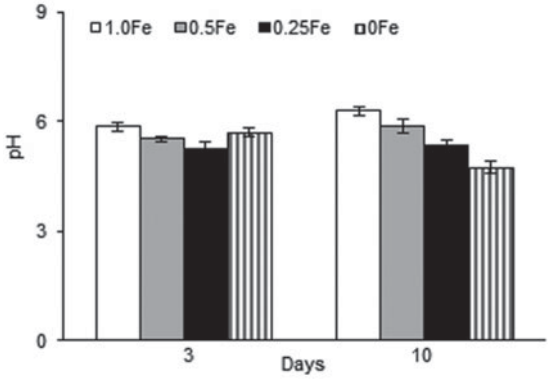

Fig. 3. Changes in pH values of the growth solution in alfalfa plants grown under decreasing Fe content. Values are means of three experiments ± S.E.

FCR activity at 3 or 10 days (Fig. 4*a*). However, under the 0Fe treatment a reduction in the FCR by 84·6% was produced only in the plants inoculated with the Ps KT2442 strain (able to produce siderophores), and not with the mutant *ppsD* (unable to produce siderophores), compared with non-inoculated plants (Fig. 4*b*).

DISCUSSION

Iron bioavailability is one of the main problems for plant growth in terrestrial ecosystems. Iron deficiency is a common agricultural problem in calcareous soils with high pH (above 6·5), affecting Fe availability. In these conditions, the crop yield is reduced due to impairments in chlorophyll biosynthesis and chloroplast development, affecting photosynthesis and many other processes where Fe enzymes or cellular Fe-dependent components are involved (Sevilla *et al*. 1984; Del Río *et al*. 1991; Hellín *et al*. 1995). Alfalfa is a model plant in phytoremediation (Peralta-Videa *et al*. 2004; Martí *et al*. 2009), and together with wheat, it is defined as less sensitive than others to metal deficiencies and specifically to Fe deficiency. The present work corroborated that alfalfa is tolerant to moderate Fe-stress conditions (0·5Fe and 0·25Fe treatments), which promoted accumulation of root and aboveground biomass without affecting Fe status or chlorophyll content (essential factors for photosynthesis and related to tolerance). However, more severe conditions (0Fe) affected the Fe content of leaves as well as the chlorophyll content. The total content of chlorophyll *a* and *b* and their ratios, which are fundamental indexes of photosynthesis activity, serves as stress indicator for plants and pollution detectors of the environment (Iturbe-Ormaetxe *et al*. 1995; Vanacker *et al*. 2006; Camejo *et al*. 2007).

In addition to the results observed in the growth parameters of the plants, at the root level alfalfa plants responded to Fe stress by decreasing the external pH

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Table 2. *Growth parameters of alfalfa plants grown for 10 days under 1·0Fe and 0Fe content in the nutrient solution in non-inoculated (control) and inoculated alfalfa seeds with* Pseudomonas putida *KT2442 and its ppsD mutant. Values are means of three experiments* ± S.E.

	Roots		Aerial part	
	Fresh weight	Dry weight	Fresh weight	Dry weight
	(g/plant)			
1·0Fe				
Control	0·07 ± 0·009	0·001 ± 0·0002	0·13 ± 0·011	0·017 ± 0·0031
Ps. KT2442	0·08 ± 0·007	0·001 ± 0·0002	0·12 ± 0·022	0·020 ± 0·0029
Ps. ppsD	0·07 ± 0·009	0·002 ± 0·0003	0·15 ± 0·023	0·025 ± 0·0032
0Fe				
Control	0·08 ± 0·012	0·007 ± 0·0001	0·15 ± 0·022	0·027 ± 0·0019
Ps. KT2442	0·06 ± 0·004	0·005 ± 0·0007	0·22 ± 0·021	0·028 ± 0·0014
Ps. ppsD	0·08 ± 0·011	0·007 ± 0·0002	0·14 ± 0·033	0·023 ± 0·0050

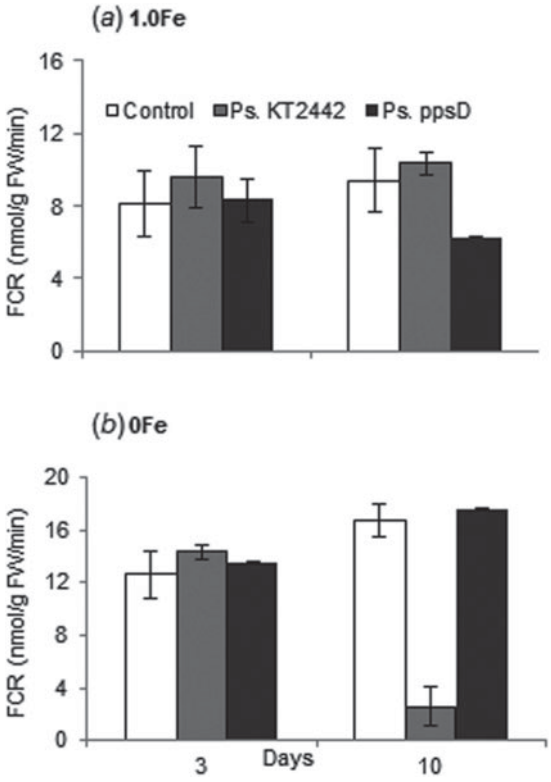

Fig. 4. Ferric chelate reductase activity of of alfalfa plants grown in 1·0Fe (*a*) and 0Fe (*b*) in the nutrient solution for 3 and 10 days in non-inoculated (control) and inoculated alfalfa seeds with *P. putida* KT2442 (Ps. KT2442) and its mutant *P. putida ppsD* (*Pseudomonas ppsD*). Values are means of three experiments ± S.E.

to increase the efficiency of Fe uptake to tolerate the stress situation. A decrease in rhizospheric pH is a response widely described in plants grown under conditions of Fe deficiency; it increases proton extrusion by the activation of a specific plasma membrane H^+-ATPase of root epidermal cells (Zocchi & Cocucci 1990; Rabotti & Zocchi 1994; Dell'Orto *et al*. 2000). In other phytoremediation species such as *Lupinus albus*, a decrease in rhizosphere pH under acid soil conditions has been described, which could be due to cation–anion balance, organic anion release, root exudation and respiration and redox-coupled processes (Hinsinger *et al*. 2003; Martínez-Alcalá *et al*. 2010).

Another strategy under Fe deficiency conditions is the observed induction of the membrane-bound ferric FCR activity of roots, which was higher at 10 days than at 3 days, indicating that absence of Fe in the nutrient solution induced this mechanism to obtain Fe from the rhizosphere, together with the acidification of extracellular media in the alfalfa plants. Previous studies found that morphological changes were induced by Fe deficiency, and an increase in lateral root branching with good correlation between total lateral root number and the Fe-deficiency-induced FCR activity has been described in red clover (*Trifolium pratense* L.) and tomato (*Solanum lycopersicum* L.) genotypes (Jin *et al*. 2008; Dasgan *et al*. 2002). In *Arabidopsis thaliana* L., genes encoding the FCR (*FRO1* and *FRO2*) and the ferrous Fe transporter (*IRT1*) have been identified and it is known that they are activated as part of the strategy I response (Robinson *et al*. 1999; Vert *et al*. 2002; Connolly *et al*. 2003). These plants released more protons to decrease the rhizosphere pH and increase Fe solubility (Morrissey & Guerinot 2009). The involvement of H^+-ATPase activity in the release of organic acids by cluster roots of white lupin (*Lupinus albus* L.), causing an acidification of the

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surrounding environment, has been demonstrated by Tomasi *et al*. (2009). The determination of FCR, which has been shown to increase under Fe deficiency stress in grass and non-graminaceous species (Robinson *et al*. 1999), has been widely used for selecting Fe-chlorosis-tolerant genotypes (Gogorcena *et al*. 2000). In contrast, phytosiderophores are used for Fe (III) chelation by graminaceous species and by bacteria and fungi (Guerinot & Yi 1994). An interesting combined strategy has been described by Cesco *et al*. (2006) in chlorosis-susceptible citrus trees (citrumelo 'Swingle' and *Citrus aurantium* L.) growing on calcareous soil, which recovered in the presence of grass species since they were able to use the Fe solubilized by the phytosiderophores released from the graminaceous species and enhancing Fe-availability. A similar strategy using the arbuscular mycorrhizal fungi *Glomus versiforme* was said to be effective by Wang *et al*. (2007); the presence of this fungi-affected citrus (*Poncirus trifoliata* L. Raf) plants' Fe uptake leading to increases in plant growth, chlorophyll content, Fe and root FCR activity. Among these mechanisms, the possibility that proliferation of bacteria, specifically Pseudomonads in the alfalfa rhizosphere, could be involved in plant responses to Fe deficiency was explored in the present work. The observed increase in the fresh weight of aboveground biomass after 10 days of Fe deficiency in plants inoculated with *P. putida* KT2442, capable of producing siderophores, is in agreement with Sharma *et al*. (2003), who observed a two-fold increase in stem fresh weight in mungbean plants inoculated with *Pseudomonas* sp. strain GRP_3. It is unlikely that the bacteria had an indirect effect through increasing the substrate pH, since carbon source concentrations in root exudates are generally below those required by the strain to produce significant pH changes through their metabolism (Romano & Kolter 2005). Furthermore, these changes would also be observed in the mutant strain and while the *P. putida ppsD* mutant strain, unable to produce siderophores, had a positive effect on the dry weight of roots and aboveground biomass of the alfalfa plants, this was only seen under control conditions of 1·0Fe. *Pseudomonas* has been reported to promote plant growth under a variety of environmental stresses, such as Fe-limiting conditions, increasing the mineral uptake by alfalfa plants and Fe availability by secreting siderophores, as described by Carrillo-Castañeda *et al*. (2002). The presence of *Pseudomonas* in the rhizosphere of the plant could promote the secretion of phytohormones and vitamins essential for growth (Mordukhava *et al*. 1991). The observed increase in FCR activity after 3 days of Fe deficiency, independently of the presence of *Pseudomonas* KT2442, indicated that the plant activated the FCR enzyme as a short-term response to Fe deficiency. However, at 10 days, inoculation with these bacteria overrode the signal induced by the deficiency, thus eliminating the FCR-mediated plant response at this time and suggesting that only conditions of prolonged absence of Fe will activate this mechanism. *P. putida* KT2440 is known to produce only one siderophore, a high Fe affinity pyoverdin with a molecular mass of 1073, the synthesis of which is controlled by several genes distributed across the genome (Matthijs *et al*. 2009). It is known that bacterial siderophores and membrane receptors are only synthesized under Fe stress conditions and the tolerance to Fe deficiency is controlled by the affinity of the siderophores for Fe, the kind and quantity of siderophores secreted, kinetics of exchange, and availability of Fe-complexes to bacteria and the plant (Negishi *et al*. 2002; Richens 2005; Dertz *et al*. 2006). Plant roots have receptors or channels that recognize the Fe^{3+}–siderophore complex; Fe^{3+} is incorporated into plants and converted to the ferrous form within the root cell (Schmidt 2003). Also, it has been reported that Fe can be removed from Fe^{3+}–siderophore complex by nicotinamine in the presence of ascorbic acid (Weber *et al*. 2008).

In order to test whether the effect of *P. putida* on FCR activity was actually due to its ability to secrete siderophores under severe Fe limiting conditions, a *ppsD* mutant derivative of *P. putida* KT2442 that is unable to produce siderophores was used. In this case, an interesting result was obtained after 10 days of Fe deficiency, when the presence of these bacteria did not produce the marked decrease in FCR activity that the wild-type *Pseudomonas* did. These results implied that bacterial influence on FCR activity and Fe uptake is due to the production of siderophores.

In conclusion, alfalfa is a crop tolerant to moderate Fe stress conditions that promote the accumulation of roots and aboveground biomass without affecting Fe status and leaf chlorophyll content. Response mechanisms focused on Fe acquisition, i.e. acidification of the external medium and activation of the FCR enzyme were induced when Fe was absent from the growth solution. The presence of *P. putida* KT2442 in the rhizosphere eliminated FCR activation under severe Fe deficiency and this effect was through the excretion of siderophores, as demonstrated using a *ppsD* mutant strain that did not produce them.

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The presence of micro-organisms able to release siderophores into the media could be one of the main benefits of the micro-organism-plant interaction in Fe deficiency conditions. Therefore, taken together with the mechanisms demonstrated to increase Fe availability, alfalfa can be recommended as a useful crop in the recovery programmes of soils where available Fe is low as consequence of soil pollution or soil degradation.

This work was supported by the SÉNECA Foundation (project no. 04553/GERM/06), DGI-FEDER (project no. BFU BFU2011-28716), EU Consolider Ingenio 2010 (no. CSD2007-0005) and Spanish Environmental Ministry (no. 116/2004/3).

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