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Abstract: Iron (Fe) deficiency is a common nutritional disorder in several crops grown in calcareous soils, but some species are well adapted to these conditions. A hydroponic experiment was conducted to compare the response of a calcicole species Ceratonia siliqua L. (carob) and of Poncirus trifoliata (L.) Raf., a citrus rootstock very sensitive to Fe deficiency. Rootstocks from both species were grown in nutrient solutions without Fe (0 μM Fe), with 1 μM Fe, and with 10 μM Fe (carob) or 40 μM Fe (P. trifoliata). A low level of Fe or its absence in the nutrient solution led to a significant decrease in P. trifoliata vegetative growth and in SPAD readings. The root activity of ferric-chelate reductase (FC-R), a key enzyme in Fe uptake, was low in the absence or with high levels of Fe. Its highest values were in roots exposed to a low level of Fe as described in several sensitive species. In contrast, the activity of FC-R was very high in carob in the absence of Fe and was decreased sharply even when only a low level of Fe was present in the nutrient solution. Plant growth and SPAD readings in the leaves of carob were similar in all treatments. Carob seems to maintain a large activity of root FC-R that may ensure enough Fe to satisfy plant demand. The fact that it presents a slow growing pattern may also contribute to the tolerance of this species to low levels of external Fe.

*Highlights

Highlight Research

A experiment was conducted to compare the response of *Ceratonia siliqua* (carob) and of *Poncirus trifoliata*, a citrus rootstock sensitive to Fe deficiency. The activity of ferric-chelate reductase (FCR) in citrus was high in low (1 µM Fe) Fe concentration and growth and SPAD readings were negatively affected. In carob, higher FCR activity was high in the absence of Fe (0 µM Fe) but with no chlorosis symptoms.

Short communication

The root ferric-chelate reductase of *Ceratonia siliqua* (L.) and *Poncirus trifoliata* (L.) Raf. responds differently to a low level of iron

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Abstract

Iron (Fe) deficiency is a common nutritional disorder in several crops grown in calcareous soils, but some species are well adapted to these conditions. A hydroponic experiment was conducted to compare the response of a calcicole species *Ceratonia siliqua* L. (carob) and of *Poncirus trifoliata* (L.) Raf., a citrus rootstock very sensitive to Fe deficiency. Rootstocks from both species were grown in nutrient solutions without Fe (0 µM Fe), with 1 µM Fe, and with 10 µM Fe (carob) or 40 µM Fe (*P. trifoliata*). A low level of Fe or its absence in the nutrient solution led to a significant decrease in *P. trifoliata* vegetative growth and in SPAD readings. The root activity of ferric-chelate reductase (FC-R), a key enzyme in Fe uptake, was low in the absence or with high levels of Fe. Its highest values were in roots exposed to a low level of Fe as described in several sensitive species. In contrast, the activity of FC-R was very high in carob in the absence of Fe and was decreased sharply even when only a low level of Fe was present

in the nutrient solution. Plant growth and SPAD readings in the leaves of carob were similar in all treatments. Carob seems to maintain a large activity of root FC-R that may ensure enough Fe to satisfy plant demand. The fact that it presents a slow growing pattern may also contribute to the tolerance of this species to low levels of external Fe.

Keywords*:* Carob-tree, Citrus, hydroponics, micronutrient, relative growth rate, rootstocks.

1. Introduction

Iron (Fe) deficiency is one of the major abiotic stresses of fruit trees in the Mediterranean area of southern Europe. The most important cause of this nutritional deficiency is the low availability of Fe to plants grown in calcareous soils (rich in lime) common in this semi-arid area. In citrus, the tolerance to Fe chlorosis is determined by the rootstock and among these *Poncirus trifoliata* (L.) Raf. is very susceptible to this deficiency (Llosá et al., 2009). The carob tree (*Ceratonia siliqua* L.) is an evergreen species present in the entire Mediterranean basin that plays an important role in the economy of several countries due to the high biotechnological value of the seeds. This crop shares the same edaphoclimatic environment as *Citru*s in southern Portugal. Under these conditions, Fe availability is similar but these two crops behave differently suggesting two different strategies to face this abiotic stress. A comparative study conducted under controlled conditions may reveal those strategies. Carob propagation in commercial orchards is achieved by grafting 2-4 year-old seedlings rootstocks. The rootstocks are obtained from seeds of female plants which are pollinated by wild, non-domesticated male trees. Field-grown carob trees, either young or mature, do not show symptoms of Fe deficiency in leaves in contrast to *Citrus* species cultivated in the same area. Moreover, its optimal growing conditions are found in calcareous, alkaline soils, i.e. it is a calcicole species (Correia and Martins-Loução, 2005).

Strategy I, found in dicots in response to Fe deficiency, includes biochemical changes with enhanced proton extrusion leading to acidification of the rhizosphere, greater activity of ferric chelate-reductase (FC-R) that convert Fe(III)-chelates to Fe(II), and more Fe(II) transporters that allows Fe to cross the root plasmalemma (Walker and Connolly, 2008).

Few studies have compared calcicole species with those sensitive to Fe deficiency. In a comparative study of two pear rootstocks, Ma et al. (2006) found that *Pyrus xerophila* Yü, a wild rootstock adapted to calcareous soils in China, showed higher values of FC-R compared to *P. betulaefolia* Bunge (used as the rootstock for the Japanese pear) when bicarbonate was added to a nutrient solution with 100 μM Fe-EDTA.

The hypothesis we tested was that carob trees, being well adapted to alkaline calcareous soils, would have developed specific mechanisms in order to overcome the detrimental effects of these soils on Fe availability and use by plants. By comparing with a non-tolerant genotype, like *Poncirus*, grown under the same conditions, it should be possible to contrast the response of the enzyme FC-R in two genetic materials. The main objective was therefore, to study key-parameters involved in this abiotic stress in order to reveal the strategy of “efficient-iron plants”.

2. Materials and methods

The experiment was conducted in a glasshouse and one-year old plants of *Ceratonia siliqua* L. (‘wild’ type) and *Poncirus trifoliata* (L.) Raf. rootstocks were transferred from NPK fertilized turf, to polystyrene boxes containing 20 L of a halfstrength Hoagland's nutrient solution with the following composition (in mM): 2.5 Ca $(NO_3)_2.4H_2O$, 2.5 KNO_3, 0.5 KH_2PO_4, 1.0 $MgSO_4.7H_2O$, and (in µM) 23.0 H_3BO_3, 0.4 $ZnSO_4.7H_2O$, 0.2 $CuSO_4.5H_2O$, 4.5 $MnCl_2.4H_2O$ and 1.0 MoO_3. Iron was added to the solutions as Fe(III)-EDDHA at three different concentrations (in µM), 0 (Fe0), 1 (Fe1) and 10 (Fe10) for *C. siliqua*, and 0 (Fe0), 1 (Fe1) and 40 (Fe40) for *P. trifoliata*, since preliminary observations indicated that 10 µM Fe was insufficient for *P. trifoliata*. The pH of the solutions was adjusted to 6.0 ± 0.1. At the beginning of the experiment, the electrical conductivity (EC) of the solution was 1.20 dS m^{-1}, and this was monitored periodically so that the solutions were changed when the value was less than 1.10 dS m^{-1}.

During the experimental period, plants were grown under natural photoperiod conditions and air temperature ≤ 25 ºC. There were 10 replications (plants) per 20 L-container, in a total of 30 plants (three containers) per treatment and each rootstock. The containers were distributed in a complete randomized design.

The shoot height was measured in all plants of each treatment at the beginning and at the end of the experiment, and to compare the two plant species, the relative growth rate (RGR) was subsequently calculated as described by Pestana et al. (2011). Total leaf chlorophyll was estimated using the portable SPAD-502 meter (Minolta Corp., Japan) in fully expanded young leaves of both species.

The activity of root FC-R (EC 1.16.1.17) was measured by the formation of the Fe(II)-bathophenantrolinedisulfonate (BPDS) complex from Fe(III)-EDTA (Bienfait et al. 1983). Measurements were performed 50 days after the beginning of the experiment, with one root tip excised with a razor blade from each plant. Each excised root tip (approximately 2 cm) was incubated in an Eppendorf tube in the dark with 900 µL of micronutrient-free half Hoagland's nutrient solution, containing 300 µM BPDS, 500 μM Fe(III)-EDTA and 5 mM MES, pH 6.0. Readings were done after centrifugation, one hour after starting the incubation. An extinction coefficient of 22.14 mM cm^{-1} was used. Blank controls without root tips were also used to correct for any unspecific Fe reduction.

The effects of Fe treatments were evaluated by one-way analysis of variance and the means compared using the Duncan Multiple Range Test (DMRT) at $P<0.05$ (SPSS software version 17.0).

3. Results

At the beginning of the experiment, carob and *P. trifoliata* plants had a height of about 15 cm and 20 cm, respectively. SPAD readings in the mature leaves were about 44 and 59 for carob and *P. trifoliata*, respectively. *P. Trifoliata* plants of the Fe 40 treatment showed the highest RGR of 12 mm per day compared to other treatments (Fe0 and Fe1). On the other hand, carob plants kept a low and constant RGR of 2 mm per day, irrespective of Fe levels in nutrient solution (Figure 1A).

At the end of the experiment only *P. trifoliata* plants grown under total Fe depletion (Fe0) or with low levels of Fe (Fe1) showed symptoms of Fe chlorosis, with SPAD values (Figure 1, A and B) of 9% and 13%, respectively, of the values of plants grown with 40 μM Fe . In contrast, SPAD readings of young carob leaves remained high in all treatments (Figure 1B) without evident symptoms of Fe chlorosis.

The highest FC-R activity (Figure 1C) in *P. trifoliata* was obtained in the Fe1 treatment, while plants of Fe40 and those grown without any Fe in the nutrient solution had lower FC-R activities. A different response was observed in carob roots, since high activity of FC-R was only observed in the Fe0 treatment.

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4. Discussion

Plants of both species growing with high levels of Fe remained green during all the experimental period, and SPAD values were within the normal range observed in Citrus (Pestana et al., 2005) and carob rootstocks (Correia et al., 2003) grown in hydroponics. Chlorotic plants with SPAD values bellow 5.0 indicate a strong decrease of leaf chlorophyll, an inefficient photosynthetic apparatus (Pestana et al., 2001) and, consequently, a small growth rate.

In contrast, carob plants grown for the same period of time (50 days) did not show symptoms of Fe deficiency even when grown with total depletion of Fe. In agreement with this, carob plants of all treatments had similar SPAD and RGR values at the end of the experiment. This means that under Fe depletion, leaf chlorophyll in newly formed leaves was ensured by Fe endogenous pools and an efficient translocation.

The differences observed between these species may be partially explained by the slow growing pattern of carob. In a recent comparative study of several Citrus rootstocks, Pestana et al. (2011) demonstrated that in Sour orange, growth rates were small and this was suggested as a strategy to explain the high degree of tolerance to Fe deficiency. Slow growing species should have smaller demands for nutrients, including Fe. Plants adapted to grow with shortage of nutrients are expected to conserve them (Lambers et al., 2008) and it is possible to presume that carob follows a conservative-type strategy.

Another key factor for the contrasting responses in both species was the different pattern of FC-R activity. There are a large number of studies demonstrating an increase of root FC-R in plants exhibiting Fe deficiency symptoms but the requirement of small amounts of Fe for FC-R has also been described in several species (e.g., Pestana et al., 2004; Abadía et al., 2011). Carob plants grown without any Fe (Fe0) had a high FC-R activity and no leaf chlorosis. Elevated Fe(III) reducing rates are related to higher tolerance to Fe stress (Castle et al., 2009) and several genes that are differentially overexpressed in Fe deficiency conditions were already identified in *P. trifoliata* (Forner-Giner et al., 2010). The high activities of FC-R may be deactivated by Fe-resupply as demonstrated by López-Millán et al., (2001). In carob we may conclude that after 50 days under total depletion of Fe in the solution (Fe0), the high FC-R activity may be considered as a response mechanism which can be an opportunity to take up greater amounts of Fe.

Since no external Fe was added to Fe0 carob plants during the 50 days of the experiment, a plant signal (endogenous Fe cannot be discarded) induced the higher FC-R activity. It is reasonable to admit that when carob plants are under severe Fe deficiency their growth is reduced to maximize the Fe-uptake mechanism (i. e. higher FC-R). In the sensitive *Poncirus*, on the other hand, a similar behaviour was observed but only if small amounts of Fe were present in the solution (Fe1). In this case, a less conservative strategy was found as the lack of Fe rapidly affected chlorophyll synthesis.

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References

Abadía, J., Vázquez, S., Rellán-Álvarez, R., El-Jendoubi, H., Abadía, A., Álvarez-Fernández, A, López-Millán, A.F., 2011. Towards a knowledge-based correction of iron chlorosis. Plant Physiol. Bioch. 49, 471-482.

Bienfait, H. F., Bino, R. J., Van der Blick, A. M., Duivenvoorden, J. F., Fontaine, J. M., 1983. Characterization of ferric reducing activity in roots of Fe-deficient *Phaseolus vulgaris*. Physiol. Plant. 59, 196-202.

Castle, W.S., Nunnallee, J., Manthey, J.A., 2009. Screening *Citrus* rootstocks and related selections in soil and solution culture for tolerance to low-iron stress. - HortScience 44, 638-645.

Correia, P.J., Pestana, M., Martins-Loução, M.A., 2003. Nutrient deficiencies in carob (*Ceratonia siliqua* L.) grown in solution culture. J. Hortic. Sci. Biotec. 78, 847-852.

Correia, P.J., Martins-Loução, M.A., 2005. The use of macronutrients and water in marginal Mediterranean areas: the case of carob-tree. Field Crops Res. 91, 1-9.

Forner-Giner, M.A., Llosá, M.J., Carrasco, J.L., Perez-Amador, M.A., Navarro, L., Ancillo, G., 2010. Differential gene expression analysis provides new insights into the molecular basis of iron deficiency stress response in the citrus rootstock *Poncuris trifoliata* (L.) Raf. J. Exp. Bot. 61, 483-490.

Lambers, H., Chapin, F.S.I., Pons, T.L., 2008. *Plant physiological ecology*. Springer, New York, USA.

Llosá, M.J., Bermejo, A., Cano, A., Quiñones, A., Forner-Giner, M.A., 2009. The citrus rootstocks Cleopatra Mandarin, *Poncirus trifoliata*, Forner-Alcaide 5 and Forner-Alcaide 13 vary in susceptibility to iron deficiency chlorosis. J. Amer. Pomolo. Sci. 63,160-167.

López-Millán, A., Morales, F., Gogorcena, Y., Abadía, A., Abadía, J., 2001. Iron resupply-mediated deactivation of Fe-deficicency stress responses in roots of sugar beet. Aust. J. Plant Physiol. 28, 171-180.

Ma, C., Tanabe, K., Itai, A., Tamura, F., Teng, Y., Chun, J.P., 2006. Responses of two asian pear rootstocks (*Pyrus* spp.) to Fe-deficiency chlorosis induced by addition of bicarbonate to nutrient solution. J. Japan. Soc. Hortic. Sci. 75, 219-223.

Pestana, M., Correia, P.J., Varennes, A. de, Abadía, J., Faria, E.A., 2001. The use of floral analysis to diagnose the nutritional status of oranges trees. J. Plant Nutr. 24, 1913-1923.

Pestana, M., Varennes, A. de, Faria, E.A., 2004. Lime-induced iron chlorosis in fruit trees. In: Dris, R., Jain, S.M. (Eds.), Production practices and quality assessment of food crops, Vol. 2: Plant mineral nutrition and pesticide management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 171-215.

Pestana, M., Varennes, A. de, Abadía, J., Faria, E.A., 2005. Differential tolerance to iron deficiency of citrus rootstocks grown in nutrient solution. Sci. Hortic. 104, 25-36.

Pestana, M., Correia, P.J., David, M., Abadía, A., Abadía, J., Varennes, A. de, 2011. Response of five citrus rootstocks to iron deficiency. J. Plant Nutr. Soil Sc. 174**,** (5): 837-846.

Walker, E.L., Connolly, E.L., 2008. Time to pump iron: iron-deficiency-signaling mechanisms of higher plants. Curr. Opin. Plant Biol. 11, 530-535.

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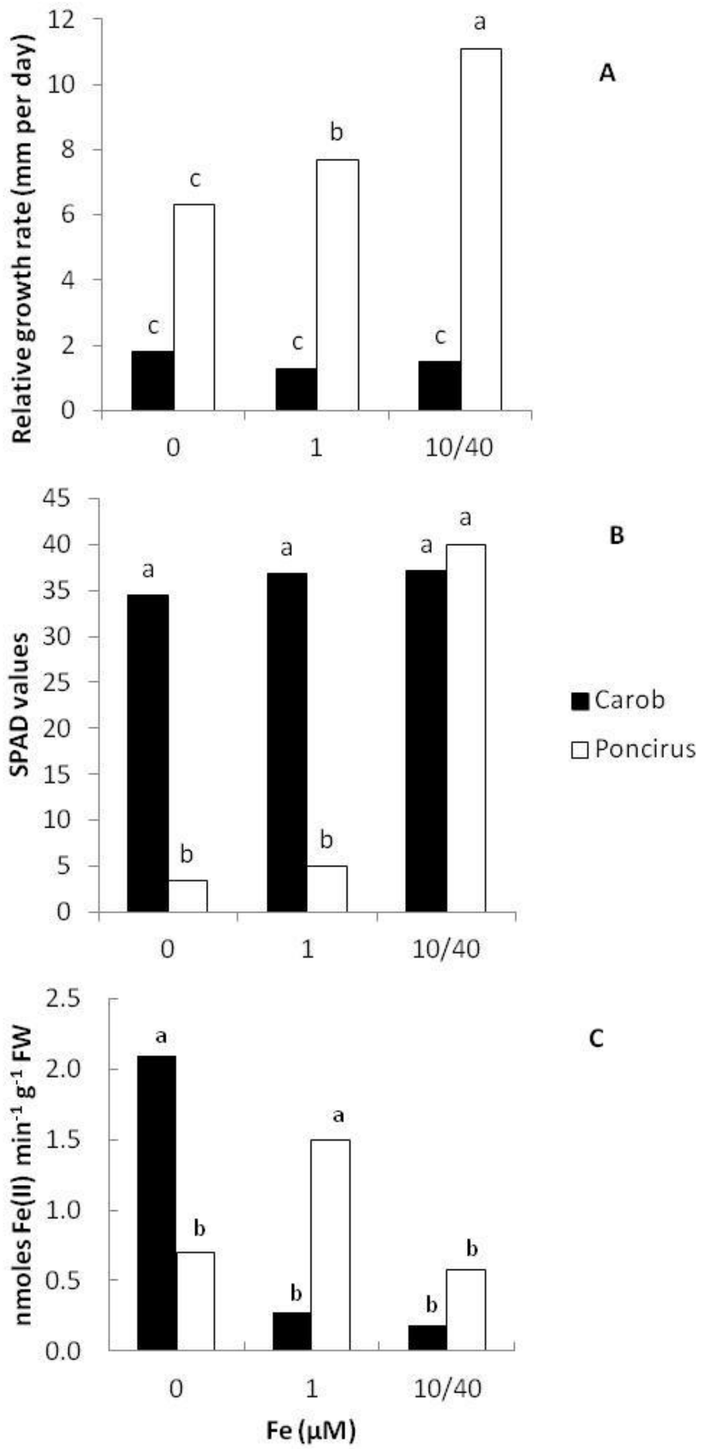

Figure 1. Relative growth rate (A), mean SPAD values (B) and FC-R activity (C) determined at the end of the experiment (after 50 days) in each treatment and plant species. In each graph, columns with different letter indicate significant differences at *P* < 0.05 (Duncan Multiple Range Test).