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Research article

Application of the split root technique to study iron uptake in cucumber plants

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ABSTRACT

The regulation exerted by the Fe status in the plant on Fe deficiency responses was investigated in *Cucumis sativus* L. roots at both biochemical and molecular levels. Besides the two activities strictly correlated with Fe deficiency response, those of the Fe(III)–chelate reductase and the high affinity Fe transporter, we considered also H^+–ATPase (EC 3.6.3.6) and phospho*enol*pyruvate carboxylase (EC 4.1.1.31), that have been shown to be involved in this response. Both enzymatic activities and gene expression were monitored using a split root system. Absence of Fe induced the expression of the four transcripts, accompanied by an increase in the corresponding enzymatic activities. The application of the split root technique gave some information about the regulation of Fe uptake. In fact, 24 h after split root application, transcripts were still high and comparable to those of the −Fe control in the Fe-supplied half side, while in the −Fe side there was a drop in the expression and the relative enzymatic activities. Major changes occurred after 48 and 72 h. The coordinated regulation of these responses is discussed.

1. Introduction

Plants require Fe to complete their life cycle. The importance of Fe is due to the existence of two stable, inter-convertible forms of this metal, which take part in fundamental processes involving electron transfer reactions, including respiration and photosynthesis [1].

Generally, there is a high quantity of Fe in the soil, but in aerobic and sub-alkaline pH environments its solubility is strongly restricted. To cope with this constraint and to enhance metal bioavailability, plants have evolved adaptation strategies to face low Fe concentrations in the environment [2]. These include morphological changes in the root architecture and specific biochemical and molecular responses serving to increase rhizosphere Fe availability and uptake [1,3].

While the uptake system through which root acquires Fe is well characterized, its regulation, as well as the flux of signals inducing or repressing these responses are not yet completely clarified. Strategy I plants (dicotyledonous and non-graminaceous) respond to lack of Fe mainly through increases in the reduction and uptake activities, by inducing trans-plasma membrane proteins in the rhizodermal cells [i.e. Fe(III)–chelate reductase [FC-R] and Iron Regulated Transporter 1 (IRT1), respectively] [1]. In Strategy I plants, an induction of genes encoding the FC-R has been observed in response to Fe starvation [4–7]. After reduction, the Fe^{II} form is taken up across the plasma membrane by a specific Fe transporter (IRT1) [7–11]. In most of the Strategy I plants, acidification of the rhizosphere occurs through the induction of a plasma membrane-located P-type H^+–ATPase [3,12–15]. A multigene family encoding different isoforms and tissue specific expression of H^+–ATPase has been demonstrated [16–19]. Moreover, significant metabolic changes occur in roots to sustain the energy requests for FC-R and H^+–ATPase activities [20]. In particular, phospho*enol*pyruvate carboxylase (PEPC) activity plays a crucial role [21,22].

Fe deficiency leads to an up-regulation of the expression of many genes involved in Fe acquisition, transport and homeostasis. Among these genes, several transcription factors in different Strategy I plants have been characterised [23 and references therein]. The regulation of genes induced by Fe deficiency is not completely elucidated, but recently new evidence has been presented to support a role for ethylene and nitric oxide in their activation [23–25]. These findings suggest that the Fe deficiency responses are modulated by a complex mechanism acting at different levels and through different signals.

In *Arabidopsis thaliana*, Vert et al. [26] showed that *IRT1* and *FRO2* are controlled both in a local and a systemic way and that these genes are over expressed during the day and down regulated at night. Despite this temporal and spatial coordination, no evidence has been reported yet about the control of the whole *iron-uptake multiple system* that also includes metabolism components [20,27].

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This work was aimed to identify the timing and coordination of biochemical and molecular responses in Strategy I plants. It was suggested [26] that two different signals, local and systemic, are involved in the regulation of the Fe status in Strategy I plants. The split-root technique allows to study the regulation of Fe-deficiency responses in cucumber roots and discriminating the roles of the systemic and localized signals involved in this regulation.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of cucumber (*Cucumis sativus* L. cv Marketmore 76) were sown in Agriperlite, watered with 0.1 mM $CaSO_4$, allowed to germinate in the dark at 26 °C for 3 d, and then transferred to a nutrient solution (30 plants/10 L) with the following composition: 2 mM $Ca(NO)_3$, 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.5 mM KH_2PO_4, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$, 0.05 μM $(NH_4)Mo_7O_{24}$ and 100 μM Fe(III)-EDTA (when added). The pH was adjusted to 6.0-6.2 with NaOH [28]. Aerated hydroponic cultures were maintained in a growth chamber with a day/night regime of 16/8 h and 24°/18 °C and a PPDF of 200 μmol m^{-2} s^{-1} at the plant level. Plants showed chlorotic symptoms after approximately seven days of culture in the absence of Fe. For split-root experiments only Fe deficient plants were used. The root system was then split into two parts and kept in separated compartments filled with a complete medium containing or not 0.1 mM Fe(III)-EDTA (Fig. 1A).

2.2. In vivo localization of the acidification and reduction capacities

Visualization and localization of acidification and Fe(III) reduction was performed by embedding the roots in an agar medium, added with the pH-sensitive dye Bromocresol Purple or with Fe^{III}–EDTA and BPDS as a chelating Fe^{II} agent, respectively.

2.3. Isolation of plasma membrane vesicles and determination of H^+–ATPase and FC-R activities

Enriched plasma membrane (PM) vesicles were obtained using the two-phase partitioning procedure as previously described [28]. Final pellets were resuspended in a medium containing 2 mM MES, pH 7.0, 1 mM PMSF and 330 mM sucrose.

H^+–ATPase activity was assayed by a spectrophotometric method, coupling ATP hydrolysis to NADH oxidation, as reported elsewhere [28]. The reaction was started by the addition of 20–50 μl aliquots of plasma membrane preparation and the absorbance changes at 340 nm were monitored over a 5 min period.

The FC-R activity was assayed at 26 °C in 1 ml volume containing 250 mM sucrose, 15 mM MOPS-BTP (pH 6.0), 0.25 mM Fe (III)-EDTA, 0.25 mM NADH and 0.01% Lubrol. The reaction was started by the addition of 20–50 μl aliquots of plasma membrane preparation and the NADH oxidation was monitored over a 5 min period at 340 nm.

2.4. PEPC assay

The enzyme was extracted from whole or split plant roots grown in the presence or in the absence of Fe as reported by De Nisi and Zocchi [21]. The reaction was started by adding aliquots of protein extract and the enzymatic assay was performed at 25 °C in 1.5 ml final volume. Oxidation of NADH was followed spectrophotometrically at 340 nm as already described.

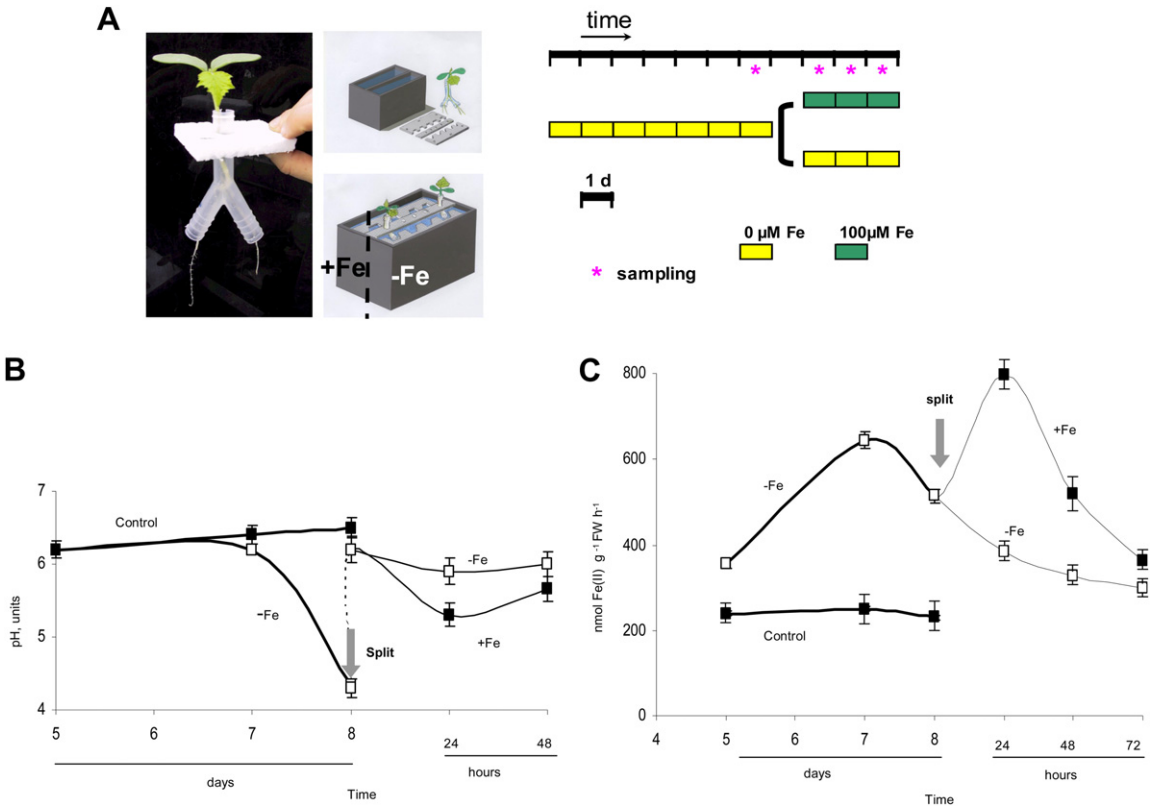

Fig. 1. Graphical representation of plant growing conditions (A) and time courses of acidification (B) and reduction (C) capacity of cucumber roots grown in the presence (closed squares) or in the absence of Fe (open squares). The split root treatment (arrows in B and C) was applied to 8-d-old Fe-deficient plants. The medium of the two compartments had the same nutrient composition plus or minus 100 μM Fe, pH 6.2.