# Reciprocal interactions between plants and fluorescent pseudomonads in relation with iron in the rhizosphere

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Iron is an essential element for plants and microbes. However, in most cultivated soils, the concentration of iron available for these living organisms is very low since its solubility is controlled by stable hydroxides, oxyhydroxides and oxides. The high demand for iron by plants and microorganisms in the rhizosphere together with its low availability in soils leads to a strong competition for this nutrient among living organisms. To face this competition, plants and microorganisms have developed active strategies of iron uptake. In non graminaceous plants (strategy I), iron uptake relies on acidification and reduction of $Fe^{+++}$ in $Fe^{++}$ which incorporated in the roots by iron transporters. Active iron uptake by microorganisms relies on siderophores showing high affinity for iron.

We have previously shown that plants of *Arabidopsis thaliana* (strategy I) supplemented with Fe-pyoverdine had a higher iron content than those supplemented Fe-EDTA [1]. Iron from pyoverdine was not incorporated through the major iron transporter IRT1 as indicated by the similar iron content of the wild-type plant and IRT1 mutant knockout iron transporter IRT1. Furthermore, pyoverdine was shown to be incorporated as indicated by its presence *in planta* based on enzyme-linked immunosorbent assay measurement of pyoverdine and on $^{15}N$ of $^{15}N$-pyoverdine. Taken together, these observations suggest that iron from Fe-pyoverdine was not incorporated *in planta* through the strategy I. In the present, we explored the possible incorporation of iron from pyoverdine at the cellular level.

For that purpose, on *Arabidopsis* when cultivated in the presence of Fe-EDTA (50µM) or Fe-pyoverdine (50µM), we analyzed the immunolocalization of pyoverdine in roots by confocal microscopy and performed ultrastructural studies with transmission electron microscopy (TEM). Plants were cultivated *in vitro* with these chelates during seven days. Immunolabeling were performed with pyoverdine antibody as primary antibody and fluorescent secondary antibody for confocal microscopy and colloidal gold coupled to secondary antibody for immunogold labeling by TEM. Observations with confocal microscopy clearly indicated the presence of pyoverdine in fresh tissue sections. This immunolocalization revealed the presence of pyoverdine in root apoplasmic space. Observations with TEM showed the more abundant presence of vesicles in root apoplasm of plants when cultured with Fe-pyoverdine than with Fe-EDTA (Figure 1). Despite that pyoverdine immunogold labeling of roots sections did not allow to reveal the formal presence

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of pyoverdine in these vesicles, the present results suggest that the incorporation of Fe-pyoverdine might rely on endocytosis.

1. G. Vansuyt et al., Mol. Plant-Microbe Interact. **4** (2007) p441.
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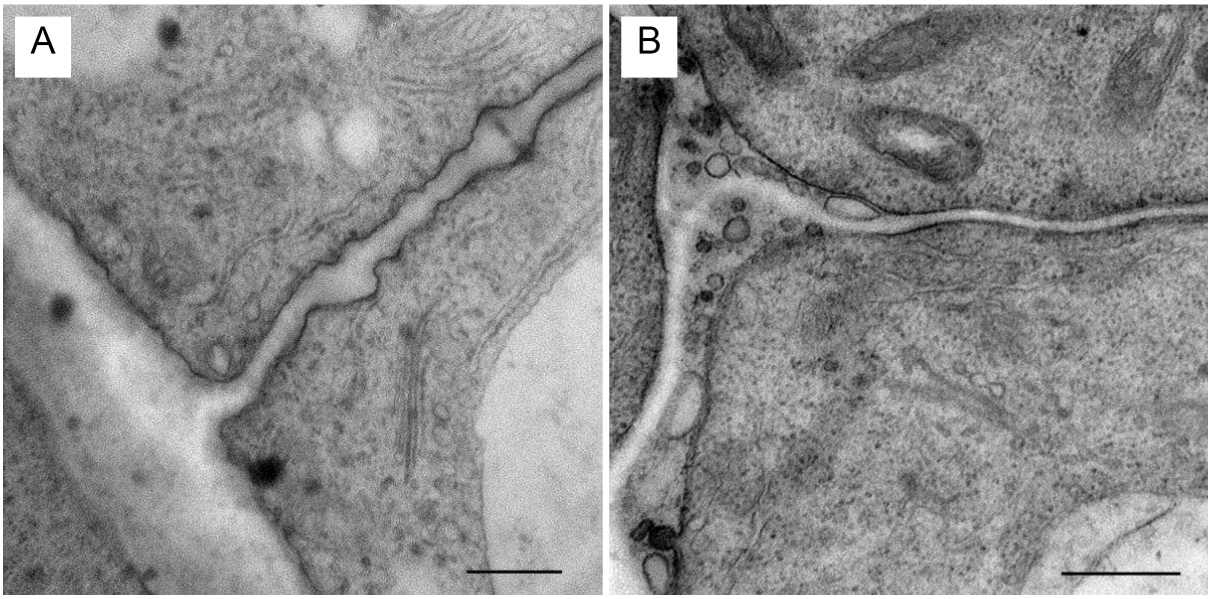


**Figure 1.** Ultrastructural observations of cells of *A. thaliana* roots supplemented with Fe-EDTA and Fe-pyoverdine imaged by transmission electron microscopy. Micrographs of root cells after seven days in presence of Fe-EDTA 50µM (A) or Fe-pyoverdine 50µM (B). Plants were grown for seven days without any iron supplementation and for seven more days after having been supplemented with 50 µM Fe-pyoverdine or Fe-EDTA or not supplemented. Bars = 500 nm.


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