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***Review*:**

Ethylene involvement in the regulation of Fe-deficiency stress responses by Strategy I plants

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Abstract. Plants have developed different mechanisms for the acquisition of iron (Fe). Depending on the mechanisms, plants are classified into two groups: Strategy I and Strategy II. Strategy I plants include all higher plants except the Gramineae, while Strategy II plants comprise the Gramineae. When plants suffer from Fe-deficiency, they develop several morphological and physiological changes in their roots, known as Fe-deficiency stress responses, which disappear when the plants acquire enough Fe. In Strategy I plants, these changes include subapical swelling with abundant root hairs, transfer cells, acidification of the rhizosphere, enhancement of the capacity to reduce Fe^{3+} to Fe^{2+}, enhancement of the capacity for Fe^{2+} uptake, release of flavins, and others. The regulation of these responses is not fully understood but in recent years there has been evidence suggesting the involvement of ethylene in this process. This review summarises different results that support a role for this hormone in the regulation of Fe-deficiency stress responses by Strategy I plants.

Keywords: auxin, ethylene, Fe-deficiency stress responses, iron, nicotianamine, regulation, Strategy I.

Introduction

Iron (Fe) is very abundant in most soils, mainly as Fe^{3+}, but its availability for plants is low, especially in calcareous soils. Based on the mechanisms developed to facilitate mobilisation and uptake of Fe, plants are classified into Strategy I plants: all higher plants except the Gramineae; and Strategy II plants: the Gramineae (Römheld and Marschner 1986; Bienfait 1987; Curie and Briat 2003; Hell and Stephan 2003). To obtain Fe from the medium, Strategy II plants release phytosiderophores from their roots, which form stable chelates with Fe^{3+}. The Fe^{3+}–phytosiderophores are then taken up by specific transporters present in the plasmamembrane of epidermal cells. Recently, one of these transporters, called YS1, has been cloned (Curie *et al*. 2001). Under Fe-deficient conditions, Strategy II plants greatly increase the production and release of phytosiderophores as well as the number of Fe^{3+}–phytosiderophore transporters (Ma and Nomoto 1996; Mori 1999). Phytosiderophores are synthesised from L-methionine via the pathway shown in Fig. 1 (Ma and Nomoto 1996; Mori 1999).

The main characteristic of Strategy I plants is the necessity for reduction of Fe^{3+} to Fe^{2+} before its absorption (Chaney *et al*. 1972). This reduction is mediated by a plasma membrane ferric reductase [encoded by the FRO1 gene in pea (Waters *et al*. 2002), and by the FRO2 gene in *Arabidopsis* (Robinson *et al*. 1999)]. When grown under Fe-deficiency, Strategy I plants induce several morphological and physiological responses in their roots, aimed at facilitating Fe mobilisation and uptake. Some of these responses are: development of subapical swelling with abundant root hairs, transfer cells, enhancement of ferric reducing capacity, acidification of the extracellular medium, increase in the number of Fe^{2+}-transporters (such as IRT1), and release of flavins and phenolics, among others (Römheld and Marschner 1986; Bienfait 1987; Curie and Briat 2003; Hell and Stephan 2003). These responses are switched on or off depending on the Fe necessities of the plant. Their regulation is not well known and the different hypotheses proposed will be briefly described in the following paragraph.

Hypotheses about the regulation of Fe-deficiency stress responses by Strategy I plants

Bienfait (1987, 1988) proposed that the FER protein is involved in the regulation of Fe-deficiency stress responses.

Abbreviations used: ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, aminooxyacetic acid; AVG, aminoethoxyvinylglycine; CFM, 2-chloro-9-hydroxyfluorenecarboxylic acid-(9)-methylester; 2,4-D, 2,4-dichlorophenoxyacetic acid; EDDHA, N,N′-ethylenebis[2-(2-hydroxyphenyl)-glycine]; Ferrozine, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine; NA, nicotianamine; SAM, S-adenosylmethionine; STS, silver thiosulfate; TIBA, 2,3,5-triiodobenzoic acid.

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The formulation of this hypothesis is based on studies with the tomato mutant *fer*. This mutant is unable to develop any of the known Fe-deficiency stress responses (subapical root hairs, transfer cells, enhancement of ferric reducing capacity, acidification) when grown under Fe-deficient conditions (Bienfait 1987, 1988; Ling *et al*. 1996). Recently, it has been found that the FER gene contained a region coding for a highly conserved bHLH motif characteristic of the family of eukaryotic bHLH transcriptional regulatory proteins (Ling *et al*. 2002). Nonetheless, it remains unclear at which point in the cascade of iron sensing, transduction of the signal or transcription of the response genes, the FER protein acts (Curie and Briat 2003).

Scholz *et al*. (1992) suggested that a nicotinamide (NA)–Fe-repressor complex participates in the repression of Fe-deficiency stress responses, in such a way that the responses are turned on when the NA–Fe-repressor complex is not formed. This hypothesis is based on the fact that the tomato mutant *chloronerva*, which lacks the ability to synthesise NA, always has the responses turned on, even when grown in Fe-sufficient conditions. This mutant becomes normal upon NA application (Scholz *et al*. 1992).

Landsberg (1984, 1996) and Römheld and Marschner (1986) proposed that Fe-deficiency increases the levels of auxin, and that this hormone triggers the Fe-deficiency stress responses. This hypothesis is based on several experimental results. TIBA (inhibitor of polar auxin transport) application to Fe-deficient plants markedly postponed the onset of acidification (Landsberg 1984), and inhibited the ferric reducing capacity and the subapical root hairs (De la Guardia *et al*. 1988). Similarly, the application of 2-chloro-9-hydroxyfluorenecarboxylic acid-(9)-methylester (CFM), another inhibitor of polar auxin transport, to Fe-deficient bean plants prevented the increase of ferric reducing capacity, although it had no effect on Fe-deficient cucumber plants (Li *et al*. 2000). On the other hand, Fe-sufficient red pepper and sunflower roots treated with auxin developed both subapical swelling with abundant root hairs and transfer cells similar to the ones developed under Fe-deficiency (Landsberg 1984, 1996). It has also been reported that Fe-deficient sunflower roots produced higher levels of auxin than the Fe-sufficient ones (Römheld and Marschner 1986).

Finally, Romera and Alcántara (1994), and Romera *et al*. (1999) proposed that Fe-deficiency causes an increase in ethylene production by roots, and that this hormone then triggers Fe-deficiency stress responses or, at least, some of them. There are several experimental results that support a role for ethylene in the regulation of Fe-deficiency stress responses in Strategy I plants. These results will be described later in this review, after a brief introduction of ethylene biosynthesis and signalling, and the role for ethylene in the responses to different stresses.

Ethylene biosynthesis and signalling

Ethylene is synthesised from L-methionine via the pathway shown in Fig. 1 (Yang and Hoffman 1984; Wang *et al*. 2002). The conversion of SAM to ACC is catalysed by ACC synthase, which is inhibited by AOA, AVG, and other compounds (Yang and Hoffman 1984). The conversion of ACC to ethylene is catalysed by ACC oxidase. ACC oxidase requires Fe for activation and is competitively inhibited by Co^{2+} (Dilley *et al*. 1993). There are also some compounds, like STS, 2,5-norbornadiene and 1-methylcyclopropene (1-MCP), that inhibit ethylene action by blocking the binding of ethylene with its receptors (Veen 1985; Hall *et al*. 2000).

The mode of action of ethylene is not fully understood, but in recent years there have been considerable advances, mainly based on the study of *Arabidopsis* ethylene mutants. Several ethylene insensitive mutants have been identified, such as *etr1*, *ein4* and *ein2*. The ETR1 and EIN4 genes encode ethylene receptors, and both mutants, *etr1* and *ein4*, show insensitivity to exogenously applied ethylene (Chang and Stadler 2001; Wang *et al*. 2002). The EIN2 gene

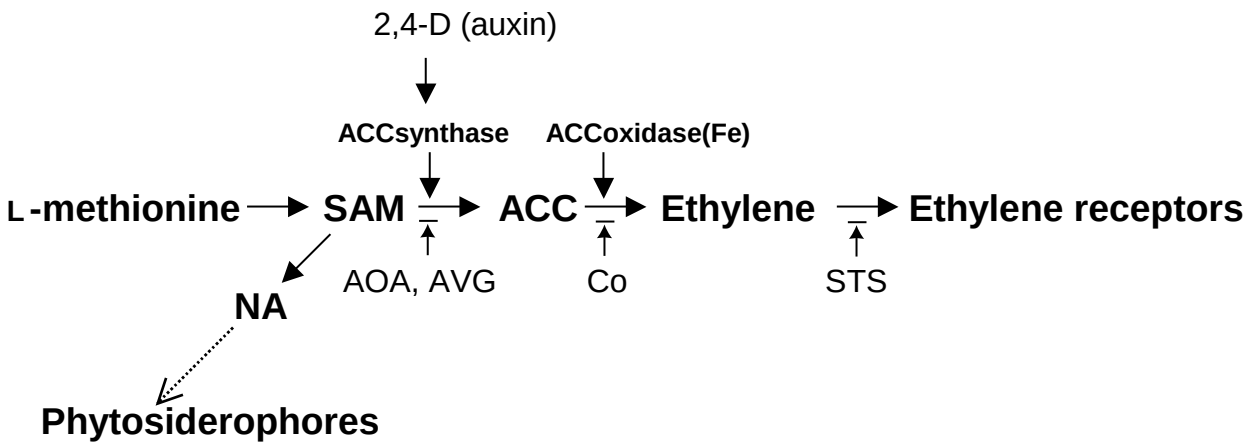

Fig. 1. Schematic pathway of ethylene biosynthesis showing the steps at which 2,4-D, AOA, AVG, Co and STS act. The pathway of NA and phytosiderophores biosynthesis is also depicted. (⋯> : only in Strategy II plants; ⊤: inhibition).

encodes a protein involved in the ethylene-signalling pathway. The e*in2* mutants also show insensitivity to exogenous ethylene (Chang and Stadler 2001; Wang *et al*. 2002). On the other hand, *ctr1* is a mutant that constitutively activates most of the responses to ethylene (i.e. triple response), as if it were always in the presence of the hormone (Chang and Stadler 2001; Wang *et al*. 2002). Based on epistatic analyses of different *Arabidopsis* ethylene mutants, a transduction pathway for ethylene has been proposed, in which CTR1 acts downstream of ETR1 and EIN4 (and other receptors: ETR2, ERS1 and ERS2), and EIN2 acts downstream of CTR1 (Chang and Stadler 2001; Wang *et al*. 2002):

$$\text{Ethylene} \rightarrow \text{ETR1, EIN4, ...} \dashv \text{CTR1} \dashv \rightarrow \rightarrow \rightarrow \text{EIN2} \rightarrow \rightarrow \text{responses.}$$

In this pathway, ethylene receptors are negative regulators of the ethylene response pathway, i.e. they repress ethylene responses in the absence of ethylene. Similarly, CTR1, which has homology to the Raf family of protein kinases, is a negative regulator of downstream signalling events (Chang and Stadler 2001; Wang *et al*. 2002).

Besides this transduction pathway, the existence of other alternative pathways is suggested by different experimental results. The *ctr1* mutants are still capable of responding to ethylene, indicating the existence of alternative pathway(s) for ethylene responses (Larsen and Chang 2001). Moreover, several monomeric G proteins, involved in signal transduction, have been shown to be transcriptionally up-regulated by ethylene, while only some of them are up-regulated in the *ctr1* mutant (Moshkov *et al*. 2003). This suggests that ethylene could act through alternative transduction pathways not mediated by the CTR1 gene (Moshkov *et al*. 2003).

Ethylene and stress

Ethylene is involved in many aspects of plant life, including seed germination, root hair development, root nodulation, flower senescence, abscission, and fruit ripening (Lynch and Brown 1997; Wang *et al*. 2002). The production of ethylene is tightly regulated by internal signals, and in response to biotic (e.g. pathogen attack) and abiotic stresses, such as wounding, mechanical stress, hypoxia, excess of ozone, chilling, or freezing (He *et al*. 1992; Morgan and Drew 1997; Wang *et al*. 2002). Stress usually promotes ethylene production (Morgan and Drew 1997).

Ethylene is also involved in the responses to different nutritional stresses, such as phosphorus (P) deficiency and Fe-deficiency (Romera and Alcántara 1994; Lynch and Brown 1997; Romera *et al*. 1999). Phosphorus-deficient roots of common bean produced more ethylene (about 2-fold) than P-sufficient ones (Borch *et al*. 1999). In addition, He *et al*. (1992) showed that nitrogen- (N) or P-deficient corn plants had enhanced sensitivity to ethylene in their roots. Barker and Corey (1988) showed that tomato plants deficient in potassium (K), calcium (Ca), or magnesium (Mg), produced more ethylene than those with no deficiencies. However, in this experiment ethylene was measured when plants showed severe symptoms of deficiency (Barker and Corey 1988), and the higher ethylene production could be due to indirect effects more than to specific effects of the deficiencies.

Ethylene and Fe-deficiency stress responses by Strategy I plants

Based on results obtained with inhibitors and precursors of ethylene, Romera and Alcántara (1994) proposed that ethylene is involved in the regulation of Fe-deficiency stress responses by Strategy I plants. These authors suggested that Fe-deficiency causes an increase in ethylene production, and consequently ethylene triggers Fe-deficiency stress responses. Later on, different experimental results have added new evidence supporting a role for ethylene in the regulation of Fe-deficiency stress responses by Strategy I plants. This evidence is based on results obtained by using different experimental approaches, such as determination of ethylene production by roots of Fe-sufficient and Fe-deficient plants, effects of ethylene inhibitors and precursors on the responses, use of mutants altered in the regulation of Fe-deficiency stress responses, and use of ethylene mutants. Here, this evidence is summarised together with a presentation of results already published by the authors and by others, and a presentation of some unpublished results from the authors.

Ethylene production by Fe-sufficient and Fe-deficient plants

Roots from several Fe-deficient Strategy I plant species produced more ethylene (up to 5-fold, depending on the species) than those from Fe-sufficient plants (Fig. 2). This higher ethylene production occurred 3–9 d after removing

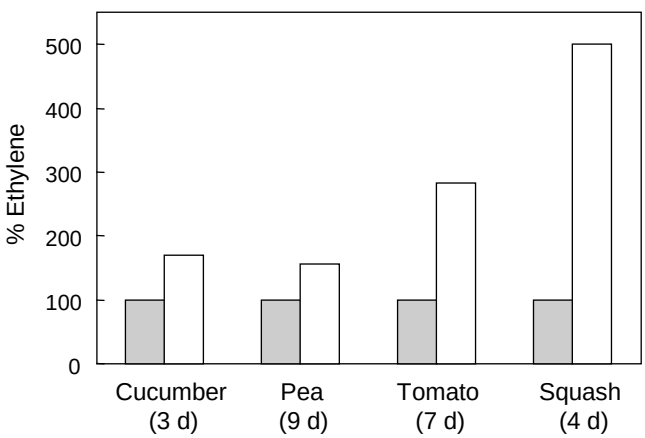

Fig. 2. Ethylene production by roots of Fe-sufficient (+Fe, filled bars) and Fe-deficient (–Fe, open bars) cucumber, pea, tomato and squash plants. Data are expressed as percentage of ethylene production in Fe-deficient plants as compared with the Fe-sufficient ones. Within parentheses is the number of days in the –Fe treatment. (Data re-elaborated from Romera *et al*. 1999 and Waters and Blevins 2000).

Fe from the nutrient solution (depending on the species), when plants still did not show severe deficiency symptoms (Romera *et al*. 1999; Waters and Blevins 2000). This observation suggests that the increase in ethylene production was due to the Fe-deficiency itself and not to an indirect effect. Moreover, the increase in ethylene production was parallel to the induction of several Fe-deficiency stress responses, such as enhanced ferric reducing capacity, subapical root hairs and acidification (Romera *et al*. 1999; Waters and Blevins 2000).

Effect of ethylene inhibitors on Fe-deficiency stress responses

The addition of inhibitors of ethylene synthesis [Cobalt (Co), AOA, AVG] or ethylene action (STS) to several Fe-deficient plants inhibited the induction of most of their Fe-deficiency stress responses (Fig. 3; Tables 1–3, 5, 6). At Co concentrations of 5 μM and lower, the values of reducing capacity achieved by several Fe-deficient Co-treated plants were under 10% of those obtained by Fe-deficient untreated plants (Table 1). Of the plant species studied, only *Arabidopsis* required higher Co concentrations (50 μM) to be inhibited (Fig. 3; Table 1). It should be noted that Co, at the concentrations used, did not cause toxic side effects, neither on plant growth nor on other visible traits. The addition of Co also inhibited other Fe-deficiency stress responses, such as acidification of the nutrient solution (Table 2), flavin excretion (Table 3), subapical root hairs, and transfer cells (Fig. 5*f*; Tables 5, 6).

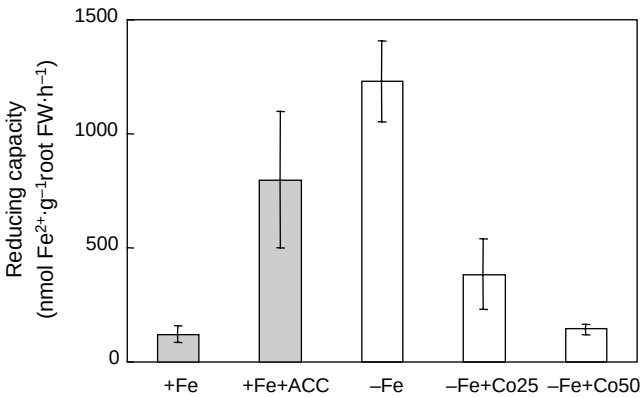

Fig. 3. Ferric reducing capacity in Fe-sufficient plants untreated or treated with ACC, and in Fe-deficient plants untreated or treated with Co, of the *Arabidopsis* mutant *etr1*. Fe-sufficient plants were grown in nutrient solution with 20 μM FeEDDHA. ACC (at 2 μM) was applied to the nutrient solution of some of the Fe-sufficient plants during 22 h before determining ferric reducing capacity. To get Fe-deficient plants, plants grown in nutrient solution with Fe were transferred to nutrient solution without Fe for 4 d. $CoSO_4$ (at 25 or 50 μM) was applied to some of the Fe-deficient plants during the last 2 d before determining ferric reducing capacity. Values are the means ± s.e. of six replicates.

As in the case of Co addition, the addition of other ethylene synthesis inhibitors to Fe-deficient plants also caused a great inhibition of ferric reducing capacity (AOA, AVG; Tables 1, 6), acidification (AOA; Table 2), flavin excretion (AOA; Table 3), and subapical root hairs (AOA, AVG; Tables 5, 6). In some plant species, at AOA

Table 1. Effect of ethylene inhibitors on the induction of ferric reducing capacity by Fe-deficient Strategy I plants
Plants were grown in nutrient solution without Fe during the last 4–5 d. Ethylene inhibitors were added to some of the plants during the last 1–2 d. Results are expressed as percentage of reducing capacity in plants treated with ethylene inhibitors relative to untreated plants

Species	Ethylene inhibitor	Concentration (μM)	% Reducing capacity in treated plants	References
Alfalfa	Co	2.5	2	Barton *et al*. 2000
Radish	Co	5	5	Heilman and Johnson 2002
Cucumber	Co	5	11	Romera and Alcántara 1994
Pea	Co	5	7	Romera *et al*. 1996*b*
Pea (*bronze*)	Co	3	13	Romera *et al*. 1996*b*
Tomato	Co	5	8	Romera *et al*. 1996*b*
Tomato (*chln*) [A]	Co	5	18	Romera *et al*. 1996*b*
Arabidopsis	Co	50	23	Romera and Alcántara 2003
Cucumber	AOA	20	29	Romera and Alcántara 1994
Pea	AOA	20	47	Romera *et al*. 1996*b*
Pea (*bronze*)	AOA	20	27	Romera *et al*. 1996*b*
Tomato	AOA	5	19	Romera *et al*. 1996*b*
Tomato (*chln*) [A]	AOA	5	28	Romera *et al*. 1996*b*
Arabidopsis	AOA	25	29	Romera and Alcántara 2003
Cucumber	AVG	10	47	Romera and Alcántara 1994
Tomato	AVG	1	140	Schikora and Schmidt 2002*b*
Cucumber	STS	800	37	Romera and Alcántara 1994
Tomato	STS	10	89	Schikora and Schmidt 2002*b*

[A]The tomato mutant *chloronerva* (*chln*) was grown with Fe (10 μM FeEDDHA).

Table 2. Effect of ethylene inhibitors on the acidification of the nutrient solution by Fe-deficient Strategy I plants

Plants were grown in small containers with 70 mL of nutrient solution without Fe, or without Fe plus an ethylene inhibitor for the last 4 d. The pH values presented correspond to the nutrient solution on the fourth day of treatment

Species	Ethylene inhibitor	Concentration (μM)	Nutrient solution pH	References
Tobacco	None	–	3.8	Landsberg 1982
	Co	20	6.2	Landsberg 1982
Cucumber	None	–	4.2	This work
	Co	5	7.2	This work
	AOA	20	7.1	This work
Tomato	None	–	4.9	This work
	Co	10	7.2	This work

concentrations of 20 μM or higher, and AVG concentrations of 10 μM, there was a slight inhibition of root growth (Romera and Alcántara 1994; Romera *et al*. 1996*b*). Nonetheless, it should be mentioned that the inhibitory effect of AVG and Co on ferric reducing capacity was reversed by the addition of ACC (Romera and Alcántara 1994) and Fe (Romera *et al*. 1996*a*), respectively, which clearly suggests that their inhibitory effect was not due to general toxic effects.

The addition of STS, an inhibitor of ethylene action (Veen 1985), to Fe-deficient plants also caused inhibition of ferric reducing capacity (Tables 1, 6) and subapical root hairs (Tables 5, 6).

In some experiments with Fe-deficient tomato plants, Schikora and Schmidt (2002*b*) did not find inhibition of reducing capacity upon treatment with either 1 μM AVG or 10 μM STS (Table 1). Probably, these AVG and STS concentrations were too low to inhibit ethylene synthesis or action in tomato. In most experiments, STS is normally used at concentrations from 0.2–2 mM (Veen 1985). Although silver is very toxic when applied as $AgNO_3$, causing high toxicity even at concentrations lower than 1 μM (Romera, unpublished results), STS has a very low toxicity (Veen 1985). It should also be noted that the concentration of ethylene inhibitor required for inhibiting ethylene production depends on the plant species and also on the growth conditions.

Effect of ethylene precursors on Fe-deficiency stress responses

In contrast with ethylene inhibitors, which blocked the induction of Fe-deficiency stress responses in Fe-deficient plants, as described in the previous paragraph, the addition of ethylene precursors to Fe-sufficient plants induced several Fe-deficiency stress responses, such as enhanced ferric reducing capacity (Figs 3, 4; Tables 4, 6), transfer cells, and subapical root hairs (Fig. 5*a*, *c*; Tables 5, 6). The addition of ACC (precursor of ethylene biosynthesis; see Fig. 1), at 0.5–1 μM concentration, to Fe-sufficient cucumber, pea, sugarbeet, *Arabidopsis*, and tomato plants enhanced their ferric reducing capacity by approximately 2–5-fold (Fig. 3; Table 4). Similarly, the addition of ethephon, an ethylene-releasing substance, to Fe-sufficient *Plantago* plants also enhanced their ferric reducing capacity approximately 2-fold (Table 4). In both cases, the enhanced reducing capacity was located in the subapical regions of the roots, where the formation of root hairs was induced, as occurred

Table 3. Effect of ethylene inhibitors on flavin excretion by Fe-deficient Strategy I plants

Plants were grown in small containers with 70 mL of nutrient solution without Fe for the last 4–7 d. Ethylene inhibitors were added to some of the plants during the last 2–3 d. Flavin concentration was determined at the end of the experiments in a Shimadzu Spectrofluorophotometer set at an activation wavelength of 445 nm (370 nm for *Arabidopsis*) and an emission wavelength of 526 nm. Riboflavin dissolved in water was used as standard. Values are the means ± s.e. of six replicates

Species	Ethylene inhibitor	Concentration (μM)	Flavin concentration (μM)	Days without Fe	References
Sugar beet	None	–	0.93 ± 0.20	7	This work
	Co	5	0.19 ± 0.05	7	This work
Cucumber	None	–	0.55 ± 0.04	4	This work
	Co	5	0.22 ± 0.04	4	This work
	Co	10	0.11 ± 0.02	4	This work
	AOA	20	0.07 ± 0.02	4	This work
Arabidopsis	None	–	0.52 ± 0.07	5	This work
	Co	50	0.01 ± 0.00	5	This work
	AOA	25	0.13 ± 0.02	5	This work

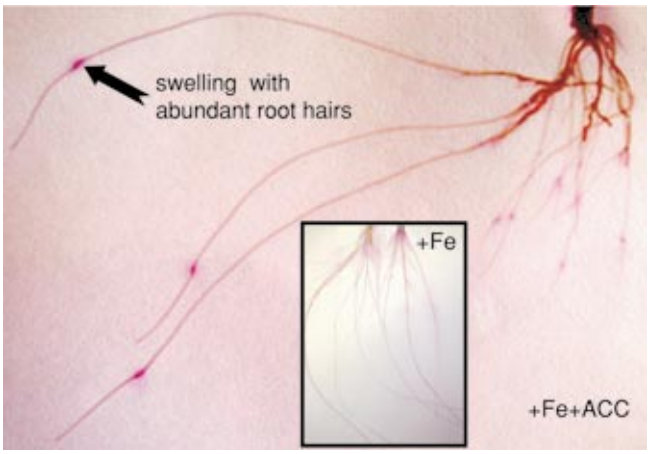

Fig. 4. Localisation of ferric reducing capacity in Fe-sufficient roots of tomato treated with ACC. Plants were grown in nutrient solution with 20 μM FeEDDHA, and 0.5 μM ACC was applied during 22 h. After that, roots were placed in agar plates with ferric reduction assay solution. Notice the red colour (due to the Fe^{2+}-ferrozine complex) around the region of subapical root hairs development. Insert shows Fe-sufficient roots not treated with ACC.

in Fe-deficient plants (Fig. 4; Romera and Alcántara 1994). It should be noted that the effect of ethylene precursors on ferric reducing capacity depended very much on their concentration and also on the duration of the treatment. When ACC and ethephon were applied in high doses (Romera *et al*. 1999) and for a long time, they did not induce ferric reducing capacity. This is logical since the responses to Fe-deficiency, like the responses to other stresses, are transitory in nature (Römheld and Marschner 1981). When ACC was applied along with Fe-deficiency to cucumber, pea, carrot, and sugarbeet plants, for 24 h, it also enhanced ferric-reducing capacity by about 2-fold (Table 4). In this latter case, the results were easier to reproduce than with Fe-sufficient plants, in which, sometimes, the addition of ACC enhanced the reducing capacity by more than 5–10-fold, while other times it had no effect. These latter results suggest that the induction of ferric reducing capacity does not depend on ethylene alone, as discussed later.

Besides ferric reducing capacity, the addition of ACC or ethephon also promoted the development of subapical root hairs in Fe-sufficient cucumber, sugarbeet, tomato (Fig. 5*a*), plantago, sunflower, *Arabidopsis* (Fig. 5*c*), soybean, and medicago plants (Tables 5, 6). Similarly, ACC and ethephon also induced the differentiation of rhizodermal transfer cells in roots of Fe-sufficient cucumber, tomato, and sunflower plants (Table 5). With regard to acidification, although we have not detected acidification of the nutrient solution (only in exceptional cases) when treating Fe-sufficient plants with ACC, we have detected acidification located in the subapical regions of the roots, when determining it in agar plates with bromocresol purple (data not shown).

Use of mutants with altered regulation of Fe-deficiency stress responses

There are some mutants with altered regulation of Fe-deficiency stress responses, such as the pea mutants *bronze* (also named *E107*) and *dgl*, the *Arabidopsis* mutant *frd3*, and the tomato mutants *chloronerva* and *fer*. The latter ones have already been described in the paragraph presenting the hypotheses about regulation. The pea mutants *bronze* (Grusak *et al*. 1990) and *dgl* (Grusak and Pezeshgi 1996), and the *Arabidopsis* mutant *frd3* (Rogers and Guerinot 2002), have some Fe-deficiency stress responses, such as enhanced ferric reducing capacity and acidification, always

Table 4. Effect of ethylene precursors on the induction of ferric reducing capacity by Fe-sufficient Strategy I plants
Plants were grown in nutrient solution with Fe. ACC or ethephon were added to some of the plants during 7–48 h. After that, reducing capacity was determined. Results are expressed as percentage of reducing capacity in plants treated with ethylene precursors relative to untreated plants

Species	Fe treatment (μM)	Ethylene precursor	Concentration (μM)	Duration of ethylene treatment (h)	% Reducing capacity in treated plants	References
Cucumber	40	ACC	1	7	275	Romera and Alcántara 1994
Cucumber	80	ACC	1	7	187	Romera and Alcántara 1994
Pea	10	ACC	0.5	7	208	Romera *et al*. 1999
Pea	40	ACC	1	48	171	Schikora and Schmidt 2002*b*
Sugarbeet	20	ACC	1	7	272	Romera *et al*. 1999
Arabidopsis	20	ACC	0.5	17	542	Romera *et al*. 1999
Tomato	20	ACC	1	22	223	Romera *et al*. 1999
Tomato	40	ACC	1	48	130	Schmidt *et al*. 2000*a*
Tomato	40	ACC	1	48	222	Schikora and Schmidt 2002*b*
Plantago	20	Ethephon	83	22	234	Romera *et al*. 1999
Cucumber[A]	0	ACC	1	24	168	Romera *et al.* 2003
Pea[A]	0	ACC	1	24	276	Romera *et al*. 2003
Carrot[A]	0	ACC	10	24	275	This work
Sugarbeet[A]	0	ACC	10	24	279	This work

[A]In some experiments, ACC was added at the same time of transferring the plants to 0 Fe treatment.

turned on, even when grown in Fe-sufficient conditions. This leads to excessive Fe accumulation and leaf toxicity (Kneen *et al*. 1990; Rogers and Guerinot 2002). Recently, it has been found that both pea mutants *bronze* and *dgl* express FRO1 (ferric reductase gene) constitutively (Waters *et al*. 2002), and the *Arabidopsis* mutant *frd3* expresses FRO2 (ferric reductase gene) constitutively (Rogers and Guerinot 2002), under Fe-sufficient conditions.

There are some experimental results suggesting that some of these mutants have alterations in ethylene metabolism or perception. In both, the pea mutant *bronze* and the tomato mutant *chloronerva*, the ferric reducing capacity, constitutively up-regulated, was drastically inhibited by addition of ethylene inhibitors (Table 1; Romera *et al*. 1996*b*). It should be noted that the *bronze* mutant exhibits a low root nodulation ability, which is partly restored upon treatment with the ethylene inhibitors AVG or Ag^+ (Guinel and LaRue 1992). Since ethylene inhibits root nodulation (Lynch and Brown 1997), it is tempting to suggest that this mutant has some alterations in ethylene metabolism or perception (Romera *et al*. 1996*b*; Guinel and Geil 2002).

The alteration in the tomato mutant *fer* is probably also related to ethylene. This mutant does not develop either transfer cells or subapical root hairs when grown under Fe-deficiency (Bienfait 1987, 1988). However, it developed both morphological responses when treated with ACC (Fig. 5*a*; Romera *et al*. 1997; Schmidt *et al*. 2000*a*). Since the FER gene probably codes for a bHLH transcription factor (Ling *et al*. 2002), the possibility exists that this transcription factor could interact somehow with ethylene.

Use of ethylene mutants

As described in the paragraph about ethylene biosynthesis and signalling, there are mutants showing insensitivity to ethylene, such as the *Arabidopsis* mutants *etr1*, *ein2*, and *ein4* (Chang and Stadler 2001; Wang *et al*. 2002); the soybean mutant *etr1* (Schmidt *et al*. 1999); and the *Medicago truncatula* mutant *sickle* (Penmetsa and Cook 1997). On the other hand, there are mutants, such as the *Arabidopsis* mutant *ctr1*, that constitutively activate most of the responses to ethylene, as if it was always in the presence of the hormone (Chang and Stadler 2001; Wang *et al*. 2002). Experiments with some of these mutants, carried out mainly by our group and the Schmidt group (Oldenburg University, Germany), have shown that ethylene is involved in the regulation of subapical root hairs. The *Arabidopsis* mutants *etr1* and *ein2* did not develop subapical root hairs either under Fe-deficiency or upon ACC treatment, while the wildtype Columbia did (Fig. 5*d*; Table 6). Similar to these *Arabidopsis* mutants, the soybean mutant *etr1* and the *Medicago truncatula* mutant *sickle*, both with insensitivity to ethylene, did not develop subapical root hairs upon ACC treatment, while their respective wild types did (Table 6). In contrast, the *Arabidopsis* mutant *ctr1* developed subapical root hairs even under Fe-sufficient conditions (Fig. 5*b*; Table 6). These results suggest that, in *Arabidopsis*, the genes ETR1, EIN2, and CTR1 are involved in the development of subapical root hairs by Fe-deficient plants. The *Arabidopsis* mutant *ein4*, also insensitive to ethylene, developed subapical root hairs either under Fe-deficiency or upon ACC treatment (Fig. 5*c*; Table 6). These results imply that the EIN4 gene, coding also for an ethylene receptor like ETR1, is not involved in the development of

Table 5. Effect of Fe and hormone treatments on the induction of transfer cells and subapical root hairs by Strategy I plants
Plants were grown in nutrient solution with or without Fe. ACC, ethephon, IAA, 2,4-D, ABA, Co, AOA, AVG or STS were added to some of the plants during several hours or days. +, promotion; –, inhibition; n.d., not determined

Species	Fe treatment	Hormone treatment	Transfer cells	Subapical root hairs	References
Cucumber	+ Fe	ACC	+	+	Romera and Alcántara 1994; Romera *et al*. 1997
Cucumber	+ Fe	2,4-D	+	+	Romera *et al*. 1997
Cucumber	+ Fe	2,4-D+Co	n.d.	–	Romera *et al*. 1997
Cucumber	+ Fe	2,4-D+STS	n.d.	–	Romera *et al*. 1997
Cucumber	– Fe	Co, AOA, AVG or STS	n.d.	–	Romera and Alcántara 1994
Cucumber	– Fe	Co	–	n.d.	Landsberg 1982
Sugarbeet	+ Fe	ACC	n.d	+	Romera *et al*. 1999
Tomato	+ Fe	ACC	+	+	Romera *et al*. 1999; Schikora and Schmidt 2002*a*; Schikora and Schmidt 2002*b;* Schmidt *et al*. 2003
Tomato	+ Fe	2,4-D	+	+	Schikora and Schmidt 2002*b;* Schmidt *et al*. 2003
Tomato (*fer*)	+ Fe	ACC	+	+	Romera *et al*. 1997; Schmidt *et al*. 2000*a*
Tomato (*fer*)	+ Fe	2,4-D	+	+	Schmidt *et al*. 2000*a*
Plantago	+ Fe	Ethephon	n.d	+	Romera *et al*. 1999
Plantago	+ Fe	2,4-D	+	+	Schmidt and Bartels 1996
Sunflower	+ Fe	Ethephon	+	+	Landsberg 1996
Sunflower	+ Fe	IAA	+	+	Landsberg 1996
Sunflower	+ Fe	ABA	–	+	Landsberg 1996

subapical root hairs by Fe-deficient plants. Additionally, these results show that different ethylene receptors could be involved in different processes.

The use of ethylene mutants to demonstrate a role for this hormone in the regulation of other Fe-deficiency stress responses, such as ferric-reducing capacity, has been more problematic and confusing. So far, all the *Arabidopsis* ethylene-insensitive mutants tested enhanced their ferric reducing capacity under Fe-deficiency (Fig. 3; Table 6; Schmidt *et al*. 2000*b*). Moreover, the *Arabidopsis* mutant

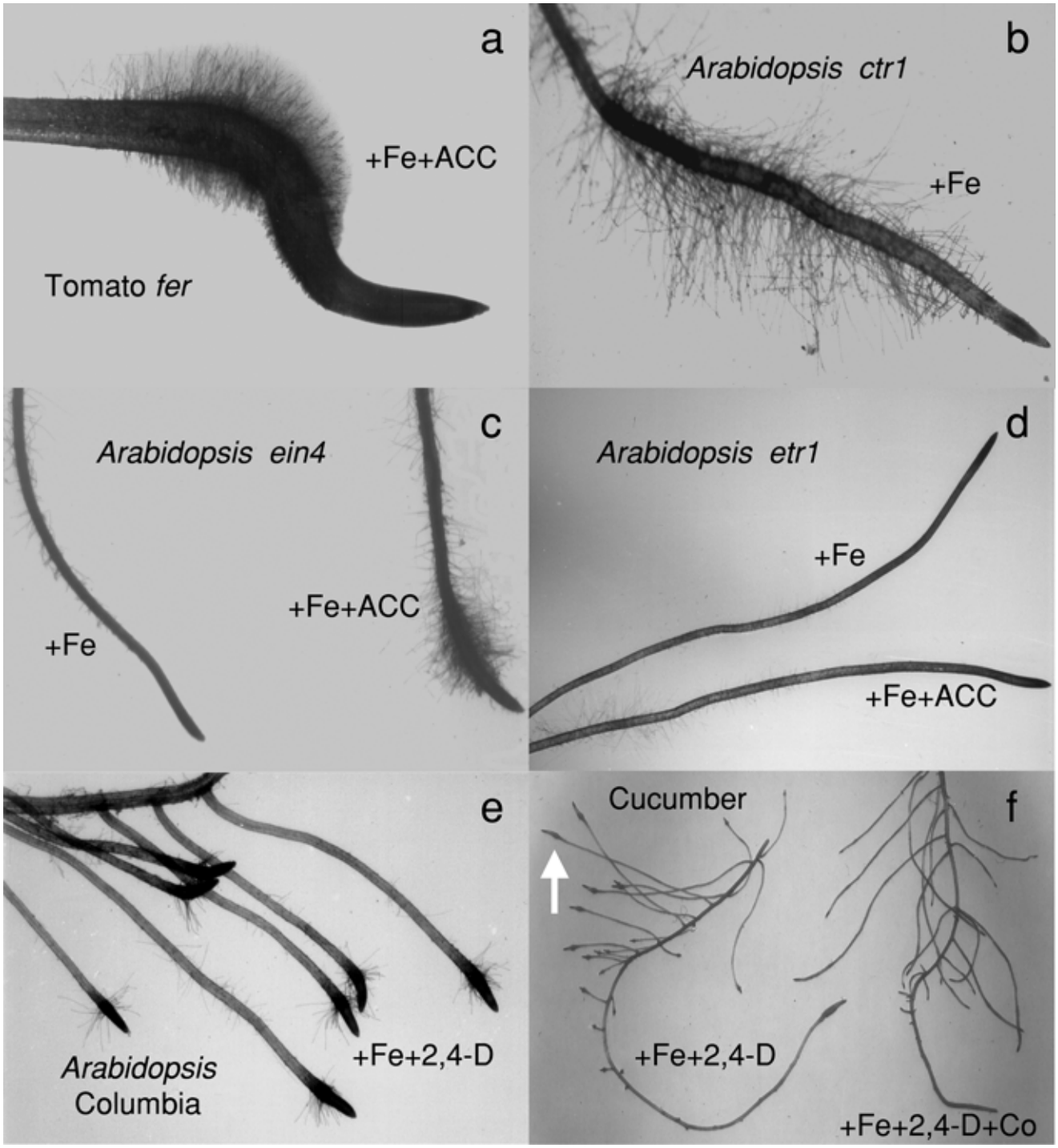

Fig. 5. Effect of ACC, 2,4-D and Co on the development of subapical root hairs by wildtype and mutant roots of different Strategy I plant species. In all cases, plants were grown in Fe-sufficient conditions, and ACC (1 μM), 2,4-D (1 μM), or $CoSO_4$ (10 μM) were added during 24 h. In Fig. 5*f*, arrow indicates swelling with abundant root hairs.

ctr1 also enhanced its ferric reducing capacity under Fe-deficiency (Table 6). The fact that the ability to enhance ferric reducing capacity is not impaired in ethylene insensitive mutants such as *etr1*, *ein2* and *ein4*; and that the *ctr1* mutant does not show constitutive high ferric reducing capacity (Table 6), have led some authors to conclude that ethylene is not involved in the regulation of ferric reducing capacity (Schmidt *et al*. 2000*b*). For us, such a conclusion is not adequate because the new knowledge about ethylene transduction and ferric reductase regulation shows that these processes are more complex than previously thought. First, the ferric reductase is regulated at both transcriptional and post-trancriptional levels (Connolly *et al*. 2003). In *Arabidopsis* transgenic plants over-expressing the FRO2 gene, FRO2 mRNA is detected at high levels in both Fe-sufficient and Fe-deficient plants. However, ferric reductase activity is only elevated in Fe-deficient plants, which suggests that FRO2 is subject to post-transcriptional regulation by Fe, as shown previously for IRT1 (Connolly *et al*. 2002, 2003). Second, there is increasing evidence that alternative ethylene transduction pathways to the one mediated by CTR1 can exist (Larsen and Chang 2001; Moshkov *et al*. 2003). Third, there is genetic evidence that the ethylene receptor family possesses partially overlapping functions (Hall *et al*. 2000), and that different ethylene receptors could form heterodimers (Wang *et al*. 2002), which implies that a mutation in one of the receptors would not necessarily block ethylene perception. Fourth, some experimental results with ethylene mutants suggest that ethylene is involved in the regulation of ferric reducing capacity. When Fe-sufficient plants of the *Arabidopsis* mutant *etr1* were treated with ACC, their ferric-reducing capacity was greatly enhanced (Fig. 3). On the other hand, the addition of ethylene inhibitors to several Fe-deficient

Table 6. Effect of Fe and hormone treatments on the induction of ferric reducing capacity and subapical root hairs by several ethylene mutants of Strategy I plants

Plants were grown in nutrient solution with or without Fe. ACC, 2,4-D, Co, AOA or STS were added to some of the plants during several hours or days. +, promotion; –, inhibition; n.d., not determined

Species	Fe treatment	Hormone treatment	Induction reducing capacity	Subapical root hairs	References
Arabidopsis (WT)	+ Fe	None	–	–	Romera and Alcántara 2003
Arabidopsis (WT)	+ Fe	ACC	+	+	Romera *et al*. 1997; Romera and Alcántara 2003
Arabidopsis (WT)	+ Fe	2,4-D	n.d.	+	Schmidt and Schikora 2001; This work
Arabidopsis (WT)	– Fe	None	+	+	Romera and Alcántara 2000; Romera and Alcántara 2003; Schmidt *et al*. 2000*b*; Schmidt and Schikora 2001
Arabidopsis (WT)	– Fe	Co, AOA or STS	–	–	Romera and Alcántara 2003; This work
Arabidopsis (*etr1*)	+ Fe	None	–	–	Romera *et al*. 1997; Romera and Alcántara 2000; Romera and Alcántara 2003; Schmidt and Schikora 2001
Arabidopsis (*etr1*)	+ Fe	ACC	+	–	Romera *et al*. 1997; Romera and Alcántara 2000; Romera and Alcántara 2003
Arabidopsis (*etr1*)	+ Fe	2,4-D	n.d.	–	This work
Arabidopsis (*etr1*)	– Fe	None	+	–	Romera and Alcántara 2003; Schmidt *et al*. 2000*b*
Arabidopsis (*etr1*)	– Fe	Co, AOA or STS	–	–	This work
Arabidopsis (*ein2*)	+ Fe	None	–	–	Schmidt *et al*. 2000*b*; Schmidt and Schikora 2001; This work
Arabidopsis (*ein2*)	+ Fe	ACC	n.d	–	This work
Arabidopsis (*ein2*)	+ Fe	2,4-D	n.d.	–	This work
Arabidopsis (*ein2*)	– Fe	None	+	–	Schmidt *et al*. 2000*b*; Schmidt and Schikora 2001; This work
Arabidopsis (*ein2*)	– Fe	Co, AOA or STS	–	–	This work
Arabidopsis (*ein4*)	+ Fe	None	–	–	Schmidt and Schikora 2001; This work
Arabidopsis (*ein4*)	+ Fe	ACC	n.d	+	This work
Arabidopsis (*ein4*)	+ Fe	2,4-D	n.d.	+	This work
Arabidopsis (*ein4*)	– Fe	None	+	+	Schmidt *et al*. 2000*b*; This work
Arabidopsis (*ein4*)	– Fe	AOA	–	–	This work
Arabidopsis (*ctr1*)	+ Fe	None	–	+	Romera and Alcántara 2000; Romera and Alcántara 2003; Schmidt *et al*. 2000*b*
Arabidopsis (*ctr1*)	– Fe	None	+	+	Romera and Alcántara 2000; Romera and Alcántara 2003; Schmidt *et al*. 2000*b*
Soybean	+ Fe	ACC	n.d	+	Schmidt *et al*. 1999; This work
Soybean (*etr1*)	+ Fe	ACC	n.d	–	Schmidt *et al*. 1999; This work
Medicago t.	+ Fe	ACC	n.d	+	This work
Medicago t. (*sickle*)	+ Fe	ACC	n.d	–	This work

ethylene-insensitive *Arabidopsis* mutants, such as *etr1*, *ein2* and *ein4*, suppressed the induction of their ferric-reducing capacity (Fig. 3; Table 6) and the development of subapical root hairs (see *ein4* in Table 6).

A conclusion easily derived from these experiments with ethylene mutants is that subapical root hairs and ferric reducing capacity are not regulated by ethylene in the same way, as first proposed by Romera *et al*. (1997). In *Arabidopsis*, ethylene could participate in the regulation of subapical root hairs through a transduction pathway including the ETR1, EIN2, and CTR1 genes, which agrees with the models proposed by Masucci and Schiefelbein (1996) and Schmidt and Schikora (2001). For the regulation of ferric reducing capacity, ethylene could act through a transduction pathway different from the one for the development of subapical root hairs (Romera *et al*. 1997; Romera and Alcántara 2000, 2003).

The ethylene hypothesis in relation to other hypotheses about regulation

In our opinion, any hypothesis about the regulation of Fe-deficiency stress responses should explain the following experimental results: (i) sometimes the responses are induced in Fe-sufficient roots; (ii) sometimes the responses are not induced in Fe-deficient roots. Both experimental results suggest that the induction of Fe-deficiency stress responses does not depend only on the root Fe content, as suggested by Bienfait *et al*. (1987), but that their regulation is more complex and probably involves some signals from the aerial part. The induction of the responses in Fe-sufficient roots was initially reported by Romera *et al*. (1992) showing, in split-root experiments, that the half root growing with Fe induced some of the responses (i.e. enhanced reducing capacity and acidification) when the other half root was growing without Fe. To explain these results, Romera *et al*. (1992) proposed the existence of a systemic signal that could move within the plant. This conclusion has subsequently been confirmed by other authors. For example, Grusak (1995) showed that the ferric reducing capacity of Fe-sufficient pea plants was modulated throughout their life cycle, which also suggests the existence of signal(s) derived from the shoot as modulators of the activity. Grusak and Pezeshgi (1996) also showed, by grafting the pea mutant *dgl* onto its wild type DGV, that the reducing capacity of the grafted plants was up-regulated, as occurred in *dgl*, which suggests that the *dgl* shoot transmits a signal compound that acts as a promoter of the reducing capacity. Other split-root experiments have also shown that the half root growing with Fe can induce some responses when the other half root is growing without Fe (Li *et al*. 2000; Schikora and Schmidt 2001; Vert *et al*. 2003).

There are also different experimental results showing that Fe-deficiency stress responses are induced more strongly in the presence of a small quantity of Fe than in its absence (see Romera *et al*. 1996*a* and references therein). In different split-root experiments it has been found that the half root growing without Fe did not induce some of the responses while the half root growing with Fe did (Li *et al*. 2000; Schikora and Schmidt 2001; Schmidt *et al*. 2003; Vert *et al*. 2003). It is important to note that in split-root experiments with *Arabidopsis*, the half root growing without Fe did not induce the expression of either FRO2 mRNA or IRT1 mRNA, which suggests that Fe is primarily required for the induction of both genes (Vert *et al*. 2003).

Although the tomato mutant *fer* has been characterised by its inability to develop any Fe-deficiency stress response when grown under Fe-deficient conditions (Bienfait 1987, 1988; Ling *et al*. 1996, 2002), recently it has been shown that it is able to induce subapical root hairs (Fig. 5*a*; Romera *et al*. 1997; Schmidt *et al*. 2000*a*) and transfer cells (Schmidt *et al*. 2000*a*) upon ACC treatment. This suggests that its inability to induce both morphological changes does not depend totally on the FER protein but in some interaction between the FER protein and ethylene. Furthermore, the FER gene is expressed in roots independently from iron-supply conditions, which suggest that FER may act together with other iron-signalling factors to respond to different Fe concentrations (Ling *et al*. 2002).

The nicotianamine hypothesis proposed by Scholz *et al*. (1992) suggested that a NA–Fe-repressor participates in the repression of the responses. If so, how can it be explained that sometimes the responses are not induced in Fe-deficient roots, in which the NA–Fe-repressor cannot be formed? There are also other experimental results that put into question this hypothesis, although they do not preclude the participation of NA in the regulation. First, the constitutive enhanced reducing capacity of the tomato mutant *chloronerva*, unable to synthesise NA, is drastically inhibited upon application of ethylene inhibitors (Table 1), which suggests that, if NA is involved in the regulation of the responses, then it would act upstream of ethylene (Romera *et al*. 1996*b*). Second, the *fer* gene is epistatic over the *chloronerva* gene (Ling *et al*. 1996), which suggests that the FER protein acts downstream of NA in the regulation of the Fe-deficiency stress responses. Since ethylene (ACC) induces morphological changes in the *fer* mutant (see the above paragraph and Fig. 5*a*), this suggests that ethylene acts downstream of both the FER protein and NA in the regulation of, at least, subapical root hairs and transfer cells.

The auxin hypothesis proposed by Landsberg (1984), as well as the ethylene hypothesis proposed by Romera and Alcántara (1994), suggests that Fe-deficiency increases the levels of these hormones, and that they trigger the responses. At first, both hypotheses could explain the induction of the responses in Fe-sufficient roots and the lack of induction in Fe-deficient roots. However, there are different experimental results that support a role for ethylene in the regulation of Fe-deficiency responses, without discarding a role for auxin,

which could act by modulating ethylene synthesis (Hansen and Grossmann 2000). Although the addition of auxin to Fe-sufficient plants induced subapical root hairs and transfer cells (see above), it is probable that this effect could be mediated by ethylene. The addition of 2,4-D (synthetic auxin) to Fe-sufficient plants induced subapical root hairs (Fig. 5*e*, *f*; Tables 5, 6), but there was no induction when it was added along with ethylene inhibitors (Fig. 5*f*; Table 5). Moreover, the addition of 2,4-D to ethylene insensitive mutants, such as *etr1* and *ein2*, did not induce subapical root hairs (Table 6), which again suggest that auxin acts through ethylene, at least in this response. The fact that other responses are also inhibited with ethylene inhibitors (Tables 1–3) suggests that, if auxin is involved in the regulation of the responses, it probably acts through ethylene.

The induction of the responses in Fe-sufficient roots, and the lack of induction in Fe-deficient roots, that occur in some conditions can be adequately explained by the ethylene hypothesis. In Fe-sufficient roots, an increase in ethylene concentration or perception could induce some responses, even in the presence of Fe. This is what happened when Fe-sufficient plants were treated with either ACC or ethephon (Figs 3, 4; Tables 4–6). In Fe-deficient roots, the lack of induction that occurs sometimes is probably related to the absence of Fe inside the roots (Vert *et al*. 2003). As discussed by Romera *et al*. (1996*a*), Fe is required for ethylene synthesis because the ACC oxidase needs Fe for its function (Dilley *et al*. 1993). In the absence of Fe, ethylene could not be synthesised and consequently the responses would not be induced. This suggestion does not discard an additional role for Fe in ethylene perception or transduction.

Are all the Fe-deficiency stress responses regulated by ethylene in the same way?

Despite evidence suggesting a role for ethylene in the regulation of Fe-deficiency stress responses by Strategy I plants, there are still some open questions. Are all the responses to Fe-deficiency regulated by ethylene? If so, are all of them regulated by ethylene in the same way? The evidence obtained by using ethylene inhibitors and precursors suggests that ethylene is involved in the regulation of most of the responses, such as reducing capacity, acidification, flavin excretion, subapical root hairs and transfer cells (Figs 3–5; Tables 1–6). However, it is possible that the different responses could be regulated by ethylene in different ways. First, the different responses could be induced upon different ethylene concentrations. In Fe-deficient pea plants, which induced reducing capacity but hardly induced subapical root hairs and transfer cells under moderate Fe-deficiency, the addition of ACC promoted the development of both subapical root hairs (Romera *et al*. 1999, 2003) and transfer cells (Schikora and Schmidt 2002*b*). This suggests that a higher ethylene concentration could be required to induce both morphological changes than to induce the reducing capacity. Similarly, reducing capacity and acidification were induced in tomato plants under moderate Fe-deficiency, while the subapical root hairs only were induced under severe Fe-deficiency (Chaney *et al*. 1992), which again suggests that this morphological change requires higher signal concentration (ethylene?) to be induced. Second, ethylene could regulate different responses through different transduction pathways. The results obtained with ethylene mutants suggest this possibility (see previous paragraph, use of ethylene mutants). As previously discussed, ethylene probably regulates the development of subapical root hairs through a transduction pathway including the ETR1, CTR1 and EIN2 genes, while it probably acts through a different transduction pathway for the regulation of reducing capacity. Third, ethylene could regulate different responses by acting in conjunction with different signals. The probable involvement of ethylene in the regulation of ferric reducing capacity and other responses does not imply that ethylene is the only signal involved in the process. Ethylene appears to be necessary for the induction of the ferric reductase, since ethylene inhibitors inhibit it and ethylene precursors enhance it (Figs 3, 4; Tables 1, 4, 6), but perhaps it is not sufficient. As previously mentioned, the ferric reductase is regulated at both transcriptional and post-transcriptional levels which would imply that, even under conditions of high FRO2 mRNA expression, the ferric reductase activity could be low, owing to its post-transcriptional regulation by Fe (Connolly *et al*. 2003). This post-transcriptional regulation would explain why the ferric reductase is not constitutively activated in the *Arabidopsis* ethylene over-producer mutant *eto* (Schmidt *et al*. 2000*b*) or in the *Arabidopsis* ethylene mutant *ctr* when grown in Fe-sufficient conditions (Romera and Alcántara 2000; Schmidt *et al*. 2000*b*). Moreover, the post-transcriptional regulation of the ferric reductase would explain why sometimes the addition of ACC or ethephon to Fe-sufficient plants had no effect on its activity, while other times the same treatment greatly enhanced it.

The regulation of other Fe-deficiency stress responses has been less studied, but results with ethylene inhibitors and precursors suggest that ethylene could also be involved in the regulation of transfer cells, flavin excretion, and acidification (Figs 3–5; Tables 1–6).

Comparison between regulation of Fe-deficiency stress responses in Strategy I and Strategy II plants

The regulation of stress responses to Fe-deficiency in Strategy II plants has been less studied than that in Strategy I plants. It is known that Strategy II plants enhance the production of phytosiderophores under Fe-deficient conditions (Ma and Nomoto 1996; Mori 1999) but, to our knowledge, no signals have been involved in their regulation. Nonetheless, some similarities could be found

between Strategy I and Strategy II plants. As shown in Fig. 1, both ethylene and phytosiderophores are synthesised from L-methionine. So, it is probable that Strategy I and Strategy II plants share some common elements in their responses to Fe-deficiency, such as alterations in the L-methionine cycle. It is tempting to suggest that during their evolution, Strategy I plants have dedicated L-methionine to synthesise ethylene, and thus respond to Fe-deficiency, while in Strategy II plants L-methionine has been dedicated to synthesise phytosiderophores. There are some experimental results suggesting ethylene is not involved in the regulation of Fe-deficiency stress responses by Strategy II plants. First, roots from several Fe-deficient Strategy II plants did not produce more ethylene than the Fe-sufficient ones, as occurred in Strategy I plants (Romera *et al*. 1999). Second, the addition of ACC to barley seedlings did not increase their production of phytosiderophores (Welch *et al*. 1997).

Concluding remarks

Our results and those of other researchers support the involvement of ethylene in the regulation of most of the Fe-deficiency stress responses by Strategy I plants. The regulation of subapical root hairs in Fe-deficient plants could be mediated by ethylene according to the following transduction pathway:

$$\text{Fe-deficiency} \rightarrow \text{ethylene} \rightarrow \text{ETR1} \dashv\| \text{CTR1} \dashv\| \rightarrow \rightarrow \text{EIN2} \rightarrow\rightarrow \text{subapical root hairs.}$$

For the regulation of the ferric reductase activity, ethylene could act through a different and unknown transduction pathway:

$$\text{Fe-deficiency} \rightarrow \text{ethylene} \rightarrow ? \rightarrow ? \rightarrow \text{FRO mRNA} \rightarrow \text{post-transcriptional regulation} \rightarrow \text{ferric reductase.}$$

The regulation of other Fe-deficiency stress responses, such as transfer cells, flavin excretion, acidification, and Fe^{2+}-transporters, have been less studied but some experimental results also suggest a role for ethylene in their regulation. Our opinion is that ethylene acts as a coordinator of most of the responses to Fe-deficiency, although in different responses ethylene could participate in different ways and, perhaps, in conjunction with other signals.

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References

Barker AV, Corey KA (1988) Ethylene evolution by tomato plants under nutrient stress. *HortScience* **23**, 202–203.

Barton LL, Johnson GV, O'Nan AG, Wagener BM (2000) Inhibition of ferric chelate reductase in alfalfa roots by cobalt, nickel, chromium, and copper. *Journal of Plant Nutrition* **23**, 1833–1845.

Bienfait HF (1987) Biochemical basis of iron efficiency reactions in plants. In 'Iron transport in microbes, plants and animals'. (Eds G Winkelmann, D van der Helm and JB Neilands) pp. 339–349. (VCH Verlagsgesellschaft mbH: Weinheim, Germany)

Bienfait HF (1988) Proteins under the control of the gene for Fe efficiency in tomato. *Plant Physiology* **88**, 785–787.

Bienfait HF, De Weger LA, Kramer D (1987) Control of development of iron-efficiency reactions in potato as a response to iron deficiency is located in the roots. *Plant Physiology* **83**, 244–247.

Borch K, Bouma TJ, Lynch JP, Brown KM (1999) Ethylene: a regulator of root architectural responses to soil phosphorus availability. *Plant, Cell and Environment* **22**, 425–431. doi:10.1046/J.1365-3040.1999.00405.X

Chaney RL, Brown JC, Tiffin LO (1972) Obligatory reduction of ferric chelates in iron uptake by soybeans. *Plant Physiology* **50**, 208–213.

Chaney RL, Chen Y, Green CE, Holden MJ, Bell PF, Luster DG, Angle JS (1992) Root hairs on chlorotic tomatoes are an effect of chlorosis rather than part of the adaptive Fe-stress-response. *Journal of Plant Nutrition* **15**, 1857–1875.

Chang C, Stadler R (2001) Ethylene hormone receptor action in *Arabidopsis. BioEssays* **23**, 619–627. doi:10.1002/BIES.1087

Connolly EL, Fett JP, Guerinot ML (2002) Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell* **14**, 1347–1357. doi:10.1105/TPC.001263

Connolly EL, Campbell NH, Grotz N, Prichard CL, Guerinot ML (2003) Overexpression of the FRO2 ferric chelate reductase confers tolerance to growth on low iron and uncovers posttranscriptional control. *Plant Physiology* **133**, 1102–1110. doi:10.1104/PP.103.025122

Curie C, Briat JF (2003) Iron transport and signaling in plants. *Annual Review of Plant Biology* **54**, 183–206. doi:10.1146/ANNUREV.ARPLANT.54.031902.135018

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature* **409**, 346–349. doi:10.1038/35053080

De la Guardia MD, Alcántara E, Fernández M (1988) Iron reduction by sunflower roots under iron stress. In 'Proceedings NATO advanced research workshop on plasma membrane oxidoreductases in control of animal and plant growth'. (Eds FL Crane, DJ Morré and H Löw) p. 430. (Plenum Press: New York, NY)

Dilley DR, Kuai J, Poneleit L, Zhu Y, Pekker Y, Wilson I, Burmeister DM, Gran C, Bowers A (1993) Purification and characterization of ACC oxidase and its expression during ripening in apple fruit. In 'Cellular and molecular aspects of the plant hormone ethylene'. (Eds JC Pech, A Latché and B. Balagué) pp. 46–52. (Kluwer Academic Publishers: The Netherlands)

Grusak MA (1995) Whole-root iron(III)-reductase activity throughout the life cycle of iron-grown *Pisum sativum* L. (Fabaceae): relevance to the iron nutrition of developing seeds. *Planta* **197**, 111–117.

Grusak MA, Pezeshgi S (1996) Shoot-to-root signal transmission regulates root Fe(III) reductase in the *dgl* mutant of pea. *Plant Physiology* **110**, 329–334.

Grusak MA, Welch RM, Kochian LV (1990) Physiological characterization of a single-gene mutant of *Pisum sativum* exhibiting excess iron accumulation. *Plant Physiology* **93**, 976–981.

Guinel FC, Geil RD (2002) A model for the development of the rhizobial and arbuscular mycorrhizal symbioses in legumes and its use to understand the roles of ethylene in the establishment of these two symbioses. *Canadian Journal of Botany* **80**, 695–720. doi:10.1139/B02-066

Guinel FC, LaRue TA (1992) Ethylene inhibitors partly restore nodulation to pea mutant E107 (brz). *Plant Physiology* **99**, 515–518.

Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB (2000) Ethylene perception by the ERS1 protein in *Arabidopsis. Plant Physiology* **123**, 1449–1457. doi:10.1104/PP.123.4.1449

Hansen H, Grossmann K (2000) Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiology* **124**, 1437–1448. doi:10.1104/PP.124.3.1437

He CJ, Morgan PW, Drew MC (1992) Enhanced sensitivity to ethylene in nitrogen-starved or phosphate-starved roots of *Zea-mays* L. during aerenchyma formation. *Plant Physiology* **98**, 137–142.

Heilman AR, Johnson GV (2002) Ferric chelate reductase in radish: response to metals and ethylene efectors. In 'Proceedings 11th international symposium on iron nutrition and interactions in plants'. p. 72. (Udine, Italy)

Hell R, Stephan UW (2003) Iron uptake, trafficking and homeostasis in plants. *Planta* **216**, 541–551.

Kneen BE, Larue TA, Welch RM, Weeden NF (1990) Pleiotropic effects of *brz. Plant Physiology* **93**, 717–722.

Landsberg EC (1982) Transfer cell formation in the root epidermis: a prerequisite for Fe-efficiency? *Journal of Plant Nutrition* **5**, 415–432.

Landsberg EC (1984) Regulation of iron-stress-response by whole plant activity. *Journal of Plant Nutrition* **7**, 609–621.

Landsberg EC (1996) Hormonal regulation of iron-stress response in sunflower roots: a morphological and cytological investigation. *Protoplasma* **194**, 69–80.

Larsen PB, Chang C (2001) The *Arabidopsis eer1* mutant has enhanced ethylene responses in the hypocotyl and stem. *Plant Physiology* **125**, 1061–1073. doi:10.1104/PP.125.2.1061

Li C, Zhu X, Zhang F (2000) Role of shoot in regulation of iron deficiency responses in cucumber and bean plants. *Journal of Plant Nutrition* **23**, 1809–1818.

Ling HQ, Pich A, Scholz G, Ganal MW (1996) Genetic analysis of two mutants affected in the regulation of iron metabolism. *Molecular and General Genetics* **252**, 87–92. doi:10.1007/S004389670010

Ling HQ, Bauer P, Bereczky Z, Keller B, Ganal M (2002) The tomato *fer* gene encoding a bHLH protein controls iron-uptake responses in roots. *Proceedings of the National Academy of Sciences USA* **99**, 13938–13943. doi:10.1073/PNAS.212448699

Lynch J, Brown KM (1997) Ethylene and plant responses to nutritional stress. *Physiologia Plantarum* **100**, 613–619. doi:10.1034/J.1399-3054.1997.1000324.X

Ma JF, Nomoto K (1996) Effective regulation of iron acquisition in graminaceous plants — the role of mugineic acids as phytosiderophores. *Physiologia Plantarum* **97**, 609–617. doi:10.1034/J.1399-3054.1996.970325.X

Masucci ID, Schiefelbein JW (1996) Hormones act downstream of *TTG* and *GL2* to promote root hair outgrowth during epidermis development in the *Arabidopsis* root. *The Plant Cell* **8**, 1505–1517. doi:10.1105/TPC.8.9.1505

Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. *Physiologia Plantarum* **100**, 620–630. doi:10.1034/J.1399-3054.1997.1000325.X

Mori S (1999) Iron acquisition by plants. *Current Opinion in Plant Biology* **2**, 250–253. doi:10.1016/S1369-5266(99)80043-0

Moshkov IE, Mur LAJ, Novikova GV, Smith AR, Hall MA (2003) Ethylene regulates monomeric GTP-binding protein gene expression and activity in *Arabidopsis*. *Plant Physiology* **131**, 1705–1717. doi:10.1104/PP.014035

Penmetsa RV, Cook DR (1997) A legume ethylene-insensitive mutant hyperinfected by its rhizobial symbiont. *Science* **275**, 527–530. doi:10.1126/SCIENCE.275.5299.527

Robinson NJ, Procter CM, Connolly EL, Guerinot ML (1999) A ferric-chelate reductase for iron uptake from soils. *Nature* **397**, 694–697. doi:10.1038/17800

Rogers EE, Guerinot ML (2002) FRD3, a member of the multi-drug and toxin efflux family, controls iron deficiency responses in *Arabidopsis. The Plant Cell* **14**, 1787–1799. doi:10.1105/TPC.001495

Romera FJ, Alcántara E (1994) Iron-deficiency stress responses in cucumber (*Cucumis sativus* L.) roots: a possible role for ethylene? *Plant Physiology* **105**, 1133–1138.

Romera FJ, Alcántara E (2000) Ferric reducing capacity and root swollen tips are differently regulated in *Arabidopsis thaliana*. In 'Proceedings 10th international symposium on iron nutrition and interactions in plants'. p. 75. (Houston, TX)

Romera FJ, Alcántara E (2003) Ethylene could be involved in the regulation of Fe-deficiency stress responses by Strategy I plants. In 'Biology and biotechnology of the plant hormone ethylene III'. (Eds M Vendrell, H Klee, JC Pech and F Romojaro) pp. 100–105. (IOS Press: Amsterdam, The Netherlands)

Romera FJ, Alcántara E, De la Guardia MD (1992) Role of roots and shoots in the regulation of the Fe efficiency responses in sunflower and cucumber. *Physiologia Plantarum* **85**, 141–146. doi:10.1034/J.1399-3054.1992.850204.X

Romera FJ, Welch RM, Norvell WA, Schaefer SC (1996*a*) Iron requirement for and effects of promoters and inhibitors of ethylene action on stimulation of Fe(III)-chelate reductase in roots of strategy I species. *Biometals* **9**, 45–50.

Romera FJ, Welch RM, Norvell WA, Schaefer SC, Kochian LV (1996*b*) Ethylene involvement in the over-expression of Fe(III)-chelate reductase by roots of *E107* pea [*Pisum sativum* L. (*brz,brz*)] and *chloronerva* tomato (*Lycopersicon esculentum* L.) mutant genotypes. *Biometals* **9**, 38–44.

Romera FJ, Alcántara E, Bartels M, Schmidt W (1997) Role of auxin and ethylene on iron stress-induced morphological changes in roots of Strategy I plants. In 'Proceedings 9th international symposium on iron nutrition and interactions in plants'. p. 31. (Stuttgart, Germany)

Romera FJ, Alcántara E, De la Guardia MD (1999) Ethylene production by Fe-deficient roots and its involvement in the regulation of Fe-deficiency stress responses by strategy I plants. *Annals of Botany* **83**, 51–55. doi:10.1006/ANBO.1998.0793

Romera FJ, Frejo VM, Alcántara E (2003) Simultaneous Fe- and Cu-deficiency synergically accelerates the induction of several Fe-deficiency stress responses in Strategy I plants. *Plant Physiology and Biochemistry* **41**, 821–827. doi:10.1016/S0981-9428(03)00117-7

Römheld V, Marschner H (1981) Rhythmic iron stress reactions in sunflower at suboptimal iron supply. *Physiologia Plantarum* **53**, 347–353.

Römheld V, Marschner H (1986) Mobilization of iron in the rhizosphere of different plant species. *Advances in Plant Nutrition* **2**, 155–204.

Schikora A, Schmidt W (2001) Iron stress-induced changes in root epidermal cell fate are regulated independently from physiological responses to low iron availability. *Plant Physiology* **125**, 1679–1687. doi:10.1104/PP.125.4.1679

Schikora A, Schmidt W (2002*a*) Formation of transfer cells and H^+–ATPase expression in tomato roots under P and Fe-deficiency. *Planta* **215**, 304–311. doi:10.1007/S00425-002-0738-0

Schikora A, Schmidt W (2002*b*) Modulation of the root epidermal phenotype by hormones, inhibitors and iron regime. *Plant and Soil* **241**, 87–96. doi:10.1023/A:1016089209891

Schmidt W, Bartels M (1996) Formation of root epidermal transfer cells in *Plantago. Plant Physiology* **110**, 217–225.

Schmidt W, Schikora A (2001) Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. *Plant Physiology* **125**, 2078–2084. doi:10.1104/PP.125.4.2078

Schmidt JS, Harper JE, Hoffman TK, Bent AF (1999) Regulation of soybean nodulation independent of ethylene signaling. *Plant Physiology* **119**, 951–959. doi:10.1104/PP.119.3.951

Schmidt W, Schikora A, Pich A, Bartels M (2000*a*) Hormones induce an Fe-deficiency-like root epidermal cell pattern in the Fe-inefficient tomato mutant *fer. Protoplasma* **213**, 67–73.

Schmidt W, Tittel J, Schikora A (2000*b*) Role of hormones in the induction of iron deficiency responses in Arabidopsis roots. *Plant Physiology* **122**, 1109–1118. doi:10.1104/PP.122.4.1109

Schmidt W, Michalke W, Schikora A (2003) Proton pumping by tomato roots. Effect of Fe-deficiency and hormones on the activity and distribution of plasma membrane H^+-ATPase in rhizodermal cells. *Plant, Cell and Environment* **26**, 361–370.

Scholz G, Becker R, Pich A, Stephan UW (1992) Nicotianamine — a common constituent of strategies I and II of iron acquisition by plants: a review. *Journal of Plant Nutrition* **15**, 1647–1665.

Veen H (1985) Antagonistic effect of silver thiosulphate or 2,5-norbonadiene on 1-aminocyclopropane-1-carboxylic acid-stimulated growth of pistils in carnation buds. *Physiologia Plantarum* **65**, 2–8.

Vert GA, Briat JF, Curie C (2003) Dual regulation of the high-affinity root iron uptake system by local and long-distance signals. *Plant Physiology* **132**, 796–804. doi:10.1104/PP.102.016089

Wang KLC, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. *The Plant Cell* **14**(Supplement), S131–S151.

Waters BM, Blevins DG (2000) Ethylene production, cluster root formation, and localization of iron(III) reducing capacity in Fe-deficient squash roots. *Plant and Soil* **225**, 21–31. doi:10.1023/A:1026513022280

Waters BM, Blevins DG, Eide DJ (2002) Characterization of FRO1, a pea ferric-chelate reductase involved in root iron acquisition. *Plant Physiology* **129**, 85–94. doi:10.1104/PP.010829

Welch RM, Norvell WA, Gesuwan P, Schaefer S (1997) Possible role of root-ethylene in Fe(III)-phytometallophore uptake in Strategy II species. *Plant and Soil* **196**, 229–232. doi:10.1023/A:1004202008059

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **35**, 155–189. doi:10.1146/ANNUREV.ARPLANT.35.1.155

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