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1 **A quick journey into the diversity of iron uptake strategies in photosynthetic**
2 **organisms**

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28 **Abstract**

29 Iron (Fe) is involved in multiple processes that contribute to the maintenance of the cellular
30 homeostasis of all living beings. In photosynthetic organisms, Fe is notably required for photosynthesis.
31 Although iron is generally abundant in the environment, it is frequently poorly bioavailable. This review
32 focuses on the molecular strategies that **photosynthetic organisms have** evolved to optimize iron
33 acquisition, using *Arabidopsis thaliana*, rice (*Oryza sativa*), and some unicellular algae as models. Non-
34 graminaceous plants, including *Arabidopsis*, take up iron from the soil by an acidification-reduction-
35 transport process (strategy I) requiring specific proteins that were recently shown to associate in a
36 dedicated complex. On the other hand, graminaceous plants, such as rice, use the so-called strategy II
37 to acquire iron, which relies on the uptake of Fe³⁺ chelated by phytosiderophores that are secreted by
38 the plant into the rhizosphere. However, apart these main strategies, accessory mechanisms
39 contribute to robust iron uptake in both *Arabidopsis* and rice. Unicellular algae combine reductive and
40 non-reductive mechanisms for iron uptake and present important specificities compared to land
41 plants. Since the majority of the molecular actors required for iron acquisition in algae are not
42 conserved in land plants, questions arise about the evolution of the Fe uptake processes upon land
43 colonization.

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47 **Keywords:** iron uptake strategies, photosynthetic organisms, iron-acquisition complex.

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57 **Introduction**

58 Iron (Fe) is essential for every form of life. Photosynthetic organisms especially need this element for
59 the electron transport chain in photosynthetic systems, which in **land** plants are located in the
60 thylakoid membranes of chloroplasts. Each photosystem I unit requires 4 FeS cluster cofactors, i.e. 12
61 Fe atoms, for functioning.¹ Plants concentrate up to 80% of cellular Fe in chloroplasts, making these
62 organelles the major Fe sinks.² In addition, Fe is needed for many key functions common to most
63 organisms such as electron transport in the respiratory chain and Fe is a cofactor in enzymes involved
64 in reactive oxygen detoxification and DNA replication, among others.³⁻⁵ On the other hand, the redox
65 properties of Fe make it potentially toxic. Fe reacts with hydrogen peroxide in the Fenton reaction to
66 generate highly toxic hydroxyl radicals. For this reason, cellular Fe accumulation must be under tight
67 control and iron has to be sequestered either in storage proteins, such as ferritins, or in organelles
68 such as the vacuole.^{6,7} Iron acquisition represents a major challenge for land plants and algae, due to
69 the exceedingly low solubility of Fe.⁸ In this minireview, we present the different mechanisms of iron
70 uptake in photosynthetic organisms that play a crucial role in the maintenance of iron homeostasis.

71

72 **Main iron-acquisition strategies of land plants: *Arabidopsis thaliana* and *Oryza Sativa* as model** 73 **organisms.**

74 Although iron is generally abundant in aerobic soils, it tends to form oxyhydrates of ferric iron (Fe^{3+}) of
75 low solubility at neutral or alkaline pH.⁹ Plants evolved two major strategies to increase the solubility
76 of immobile iron pools. All **land** plant species except the grasses use a three-step process, called
77 strategy I, based on the acidification-reduction-transport triad,¹⁰ that has been well described in the
78 model plant *Arabidopsis thaliana* (Fig. 1A). Briefly, soil acidification facilitates the dissolution of Fe^{3+}
79 precipitates and increases iron availability by several orders of magnitude. The subsequent reduction
80 of ferric chelates weakens the stability of the chelates and releases ferrous iron (Fe^{2+}). Fe^{2+} is finally
81 taken up by an iron transporter into root cells.^{7,11} This strategy is strongly inhibited in soils with high
82 pH or with high bicarbonate levels. In calcareous soils, which represent 30% of arable land, the protons
83 released by iron-deficient plants are buffered by bicarbonates^{11,12} and ferric chelate reduction is
84 strongly impaired.¹³⁻¹⁵ Gramineous plants, such as *Oryza sativa*, use the so-called strategy II to take
85 up iron (Fig. 1B), relying on the release in the soil of mugineic acid-type, hexadentate chelators, called
86 phytosiderophores.^{16,17} These organic compounds bind Fe^{3+} and are taken up as intact Fe^{3+} -
87 phytosiderophore complexes without the requirement of a reduction step.¹⁸ In contrast to reduction-
88 based Fe acquisition developed by strategy I plants, phytosiderophore-dependent Fe chelation and
89 uptake are largely insensitive to high soil pH.¹⁰

90 **Mechanisms for iron acquisition in *Arabidopsis thaliana***

91 ***The core of the iron uptake machinery***

92 Extensive study of the model strategy I plant *Arabidopsis thaliana* revealed the identity of the genes
93 encoding the core components of the acidification-reduction based strategy (Fig. 1A). Rhizosphere
94 acidification is mediated by proton extrusion by the AHA family of P-type H⁺-ATPase. In roots, the
95 *Arabidopsis* plasma membrane H⁺-ATPase 2 (AHA2) is the major ATPase isoform¹⁹ and has been shown
96 to be expressed in epidermal cells including root hairs, in the cortex, and in phloem and xylem
97 parenchyma cells.^{20,21} Although AHA2 clearly participates in many biological functions in plants, the
98 fact that *AHA2* transcripts accumulate to higher levels under iron-deficient conditions clearly argues
99 for its contribution to the acidification-driven iron uptake process. Accordingly, loss of *AHA2* function
100 impairs proton extrusion capacity upon iron shortage. The reduction of soluble ferric iron is carried out
101 by the Ferric Reduction Oxidase 2 (FRO2). The *ferric reductase defective-1* or *frd1* mutant, defective in
102 the *FRO2* gene, is impaired in iron deficiency-induced ferric chelate and copper reductase activity,
103 accumulates less iron and is severely chlorotic.²² Since *FRO2* expression is regulated by iron but not
104 copper,^{23,24} the major function of FRO2 appears to be associated with iron uptake. FRO2 transfers
105 electrons from NADPH in the cytoplasmic side via flavin and two heme groups to apoplastic Fe³⁺, thus
106 producing Fe²⁺.²⁵ Reduced iron is then taken up by the root epidermis-expressed metal transporter
107 Iron Regulated Transporter 1 (IRT1). IRT1 is poorly selective and mediates the uptake of other divalent
108 non-iron metals such as zinc, manganese, cobalt or cadmium.^{26–30} The fact that among all these metals
109 only iron regulates *IRT1* transcription points to the specific involvement of IRT1 to iron uptake,³⁰ with
110 other metals being non-specifically transported during this process. As a consequence, an *irt1* knock-
111 out mutant is strongly chlorotic due to low iron accumulation in plant tissues and is defective for the
112 low iron-induced accumulation of zinc, manganese or cobalt.³⁰ Interestingly, the expression of the
113 genes encoding the core of the iron uptake machinery, i.e. *IRT1*, *FRO2* and *AHA2*, is activated under
114 iron-limited conditions by the same basic Helix-Loop-Helix (bHLH) transcription factor called FER-like
115 Iron Deficiency-Induced Transcription Factor (FIT) that can form heterodimers with other bHLH
116 proteins.^{31–33}

117 ***Coumarins contribute to robust iron uptake in Arabidopsis***

118 As mentioned above, the acidification and FRO-dependent reduction perform rather poorly in alkaline
119 soils, and are assisted by a second shell of mechanisms that contribute to robust iron uptake. The
120 secretion of phenolic compounds, organic acids, flavonoids, and flavins has also been involved in the
121 acidification-reduction strategy to take up iron.^{34–39} In particular, a class of coumarin-type siderophores
122 derived from the phenylpropanoid pathway assists the membrane-bound acidification and ferric

123 reduction by solubilizing and reducing iron from insoluble sources (Fig. 1A). pH modulates the
124 biosynthesis of coumarins, with the main catechol coumarin fraxetin being produced at alkaline pH
125 while acidic pH favors sideretin.⁴⁰ Coumarin biosynthesis requires the Feruloyl coenzyme A 6'-
126 hydroxylase 1 (F6'H1) enzyme, whose gene is induced upon low iron condition.³⁸ Mutants defective in
127 F6'H1 are chlorotic and more sensitive to iron deficiency,³⁸ highlighting the role of coumarins in iron
128 uptake. Coumarins are secreted in the rhizosphere through the ABC-type transporter ABCG37/PDR9
129 (Fig. 1A), and *ABCG37/PDR9* expression is also boosted by iron shortage.⁴¹ Besides their direct role in
130 increasing iron availability, coumarins also modify the root microbiota-mediated iron solubilization to
131 further exploit low iron sources in the soil. The latter role of coumarins again depends on the plant
132 iron uptake machinery.⁴² In addition to IRT1, other metal transporters likely contribute to iron
133 acquisition from the soil. The Natural Resistance-Associated Macrophage Protein 1 (NRAMP1) metal
134 transporter is upregulated under Fe deficiency and behaves as a low affinity iron transporter, backing
135 up IRT1 when iron concentrations are suboptimal.^{43,44}

136

137 ***Spatial regulation of iron uptake at the plasma membrane***

138 Besides the preferred transcription of *FRO2* and *IRT1* genes in root epidermal cells under iron-limited
139 conditions, several post-translational events target the core of the iron uptake machinery. The first
140 mechanism involves the degradation of IRT1 when plants face an excess of zinc, manganese or cobalt.
141 Contrary to iron, these metals do not require prior reduction by FRO2 to be transported by IRT1. Upon
142 high influx of zinc, manganese or cobalt through IRT1, these non-iron metals are sensed by a histidine-
143 rich motif in a large cytosolic loop of IRT1 that is likely sitting at the exit of the metal permeation
144 domain.⁴⁵ Non-iron metal binding to this histidine-rich motif allows the recruitment of the Calcineurin
145 B-like (CBL)-interacting serine/threonine-protein kinase 23 (CIPK23) and phosphorylation of
146 neighboring serines and threonines. These act as a docking site for the E3 ubiquitin ligase IRT1
147 DEGRADATION FACTOR1 (IDF1) that mediates the K63 polyubiquitination of IRT1 and its endocytic
148 trafficking to the vacuole for degradation.⁴⁵ This non-iron metal-dependent degradation of IRT1 takes
149 place in soil patches rich in zinc, manganese or cobalt and limits the **acquisition** of these potentially
150 toxic elements, while IRT1 safely takes up iron where non-iron metals are low. **Besides CIPK23 and**
151 **IDF1, the peripheral membrane protein ENHANCED BENDING1 (EHB1) was demonstrated to interact**
152 **with and negatively regulate IRT1 in a calcium-dependent manner, leading to a reduction of iron**
153 **acquisition in plant.**⁴⁶ **One hypothesis proposed by Khan and co-workers is that EHB1 might be**
154 **implicated in IRT1 endocytosis, as suggested by an increase in IRT1 protein content in *ehb1* mutants**
155 **and the fact that proteins from the same family can induce membrane tubulation in vitro.**

156 In addition to the regulated trafficking of IRT1, recent data suggest that the formation of a protein
157 complex involving AHA2-FRO2-IRT1 may also be important to optimize Fe uptake at the plasma
158 membrane (Fig. 1A).⁴⁷ All three proteins were demonstrated to co-localize at the cell surface. AHA2
159 shows an even distribution while both FRO2 and IRT1 display a polar localization, being enriched at the
160 outer plasma membrane domain facing the rhizosphere.^{45,47,48} The close proximity of FRO2 and IRT1
161 may allow the funneling of iron by coupling reduction and transport. This complex likely facilitates iron
162 uptake in the aerobic soil environment, by limiting the re-oxidation of FRO2-produced ferrous iron.
163 The association of AHA2 may create a local acidic pH environment in the vicinity of FRO2 to avoid the
164 detrimental effects of high pH or bicarbonates on ferric reduction. The functional relevance of such
165 protein complex is still unclear and will await the identification of factors or residues in AHA2-FRO2-
166 IRT1 important for its formation. Whether AHA2, FRO2 and IRT1 form an obligate protein complex for
167 efficient iron transport remains to be determined. Interestingly, overexpression of IRT1 or FRO2 alone
168 can increase Fe uptake.^{23,49} The fact that both FRO2 and IRT1 are limiting for iron **acquisition** argues
169 against a scenario in which a stoichiometric complex between AHA2, FRO2 and IRT1 is required for
170 efficient Fe uptake. This suggests that a pool of free FRO2 or IRT1 localized at the plasma membrane
171 may contribute to iron import into root epidermal cells. Until now, FRO2 and IRT1 were reported to
172 strictly co-localize at the outer plasma membrane domain of root epidermal cells.⁴⁷ The limit of
173 resolution of confocal microscopes however prevents us from reaching definitive conclusions. The
174 development of super-resolution imaging approaches with FRO2 and IRT1 will certainly help visualize
175 free and complex-loaded FRO2 and IRT1 proteins at the cell surface. Regardless, the stability of the
176 AHA2-FRO2-IRT1 complex is regulated by non-iron metal transport. Indeed, elevated levels of zinc,
177 manganese or cobalt trigger IRT1 internalization and vacuolar degradation, while AHA2 and FRO2 seem
178 to be largely unaffected.⁴⁷ The disassembly of the complex is driven by CIPK23-mediated
179 phosphorylation of IRT1 upon non-iron metal excess. The fate of the released AHA2 and FRO2 has not
180 been determined but they likely engage in other processes at the cell surface. AHA2 being central to
181 proton extrusion most likely participates to many other cellular processes while FRO2 may contribute
182 to copper reduction.

183 The existence of the AHA2-FRO2-IRT1 complex raises the possibility that additional proteins involved
184 in iron uptake may also be found in a higher order protein complex. The Feruloyl-Coenzyme A 6'-
185 Hydroxylase 1 (F6'H1) coumarin biosynthetic enzyme and the ABC-type transporter ABCG36/PDR8, a
186 close homolog of the PDR9 coumarin efflux transporter, were identified in IRT1 interactome.⁴⁷ Both
187 PDR9 and PDR8 are found in the outer plasma membrane domain of root epidermal cells,⁵⁰ thus
188 coexisting in the same polar domain as FRO2 and IRT1. Although PDR9 was demonstrated to secrete
189 coumarins for iron nutrition, PDR8 was reported to export various molecules including antimicrobial

190 metabolites, cadmium or indole 3-butyric acid.^{51–55} However, considering that ABC transporters usually
191 show overlapping substrate specificity, even for root exudation,⁵⁶ the possibility that PDR8 is also
192 involved in exudation of iron uptake-related coumarins should be considered. Metabolite profiling of
193 *pdr8* and *pdr9* mutants however suggest that PDR9 but not PDR8 transport phenolic compounds,
194 including coumarin.⁵⁷ Considering that the low affinity iron transporter NRAMP1 also uses ferrous iron
195 as substrate, it may also associate with FRO2 for efficient iron transport. The exciting possibility that a
196 higher order protein complex gathering the major actors of the primary iron uptake machinery and the
197 accessory proteins in an acidification-reduction-transport platform dedicated to iron uptake will
198 deserve more attention in the future.

199 **Rice combines two strategies for iron acquisition**

200 Rice (*Oryza sativa*) secretes 2'-Deoxymugineic acid (DMA) to chelate Fe³⁺ from the soil and increase its
201 solubility. DMA is synthesized from S-adenosylmethionine through three successive enzymatic
202 reactions catalyzed by nicotianamine synthase (NAS),⁵⁸ nicotianamine aminotransferase (NAAT)⁵⁹ and
203 deoxymugineic acid synthase (DMAS).⁶⁰ The expression of *NAS*, *NAAT* and *DMAS* genes is largely
204 induced under iron deficiency in roots. DMA are then secreted in the rhizosphere by Transporter Of
205 Mugineic acid family phytosiderophores 1 (TOM1) (Fig. 1B). When expressed in *Xenopus* oocytes,
206 TOM1 behaves as a DMA efflux transporter.¹⁷ In rice, *TOM1* is expressed in root in response to iron
207 deficiency and its overexpression and silencing lead to an increase and a decrease of DMA secretion,
208 respectively. Consistently, plants overexpressing TOM1 are more tolerant to iron deficiency.¹⁷ Apart
209 from TOM1, another efflux transporter named Phenolics Efflux Transporter 2 (PEZ2) was proposed to
210 be involved in rice iron acquisition by exporting phenolic compounds such as protocatechuic acid and
211 caffeic acid in the rhizosphere to increase iron solubility.⁶¹ In graminaceae, protocatechuic acid may
212 thus play a similar role to solubilize Fe as fraxetin, esculetin and sideretin in dicots. After iron chelation,
213 Fe³⁺-DMA complexes are transported into root epidermal cells by a Yellow Stripe1-Like (YSL) protein
214 named OsYSL15 (Fig. 1B). *OsYSL15* gene is mostly expressed in root epidermis/exodermis and phloem
215 cells under iron deficiency and its knockdown leads to severe defects in germination and early seedling
216 growth that are reverted by iron supply.⁶² In addition, insertional *osys15* mutants were shown to
217 exhibit reduced iron concentrations.⁶³ Other members of the OsYSL family may be implicated in iron
218 uptake from the rhizosphere. Thus, OsYSL16 protein transports Fe³⁺-DMA as revealed by a
219 complementation assay performed on the yeast *fet3fet4* mutant defective in iron uptake and *OsYSL16*
220 gene is expressed in the rice root epidermis, but contrary to *OsYSL15*, independently of the iron status
221 of the plant.⁶⁴ This suggests that rice combines a constitutive and an inducible component for Fe-
222 siderophore complex uptake.

223 Although rice is considered as a strategy II plant for iron uptake, it also uses mechanisms from strategy
224 I. Indeed, rice was demonstrated to directly absorb Fe^{2+} from the soil in addition to Fe^{3+} -
225 phytosiderophore complexes (Fig. 1B).⁶⁵ This process is likely mediated by the OsIRT1 and OsIRT2
226 transporters since: (i) similarly to other iron acquisition components, *OsIRT1* and *OsIRT2* are expressed
227 in roots under iron deficiency, (ii) both proteins are localized in the plasma membrane, (iii) OsIRT1 and
228 OsIRT2 transport Fe^{2+} as revealed by yeast complementation assays.^{65,66} This second system allowing
229 Fe^{2+} acquisition would be in accordance with the fact that rice secretes relatively low amount of
230 phytosiderophores compared to other graminaceous plants.⁶⁷ Contrary to the *Arabidopsis* iron
231 acquisition strategy, the activity of FRO ferric reductase seems to be dispensable for Fe^{2+} uptake under
232 iron deficiency in rice, suggesting that OsIRT1 works independently of FRO proteins.⁶⁵ In paddy fields,
233 where rice is grown, Fe^{2+} is abundant due to the low redox potential and therefore rice plants do not
234 need to reduce Fe^{3+} to Fe^{2+} . However, Ishimaru and co-workers showed that enhancing the root Fe^{3+}
235 chelate-reductase activity of rice plants by expressing the mutational reconstructed yeast Fe^{3+} chelate-
236 reductase gene *refre1/372*, under the control of *OsIRT1* promoter, conferred resistance to low iron
237 availability on calcareous soils.⁶⁸ The combined strategy for iron acquisition is not specific to *O. sativa*
238 but is also present in wild species of the *Oryza* genus, demonstrating that the adaptation to Fe^{2+} uptake
239 in flooded soils precedes *O. sativa* domestication.⁶⁹ Some authors recently proposed that, apart from
240 rice, other graminaceous plants such as maize may combined strategy II and some features of
241 archetypal strategy I system for iron acquisition.⁷⁰

242

243 **An overview of Fe uptake strategies in different organisms related to plants**

244 The origin of the diversity of Fe uptake systems found in land plants may be traced back in unicellular
245 algae. Iron is also a major limiting factor for the growth of phytoplankton.⁷¹ Iron solubility depends on
246 pH, carbonate concentration and temperature.⁷² Fe is scarcely available in well oxygenated water and
247 most of the iron is bound to organic compounds or colloid particles of oxyhydroxides, as in soils. To
248 cope with the extreme scarcity of this element and adapt to the diversity of Fe sources and their
249 changes according to environmental factors, unicellular algae have evolved a plethora of iron
250 acquisition systems that often coexist in the same species. This is well illustrated by the study of Fe
251 uptake systems in the two main algal models: *Chlamydomonas reinhardtii* and the diatom
252 *Phaeodactylum tricornutum*. They both possess reductive and non-reductive strategies for iron
253 acquisition (Fig. 1C). Interestingly, when reduction-based iron uptake coexists with another iron
254 acquisition system, the expression of the genes involved in the different iron acquisition strategies are
255 co-regulated, as demonstrated in *C. reinhardtii* but also in rice by analyses of transcriptomic data.⁷³ For

256 non-reductive uptake, *P. tricornutum* and *C. reinhardtii* use a transferrin-like (phyto-transferrin)
257 protein, named Iron Starvation Induced protein 2A (ISIP2A) and Fe-assimilating protein 1 and 2 (FEA1
258 /FEA2), respectively, that bind Fe³⁺ in the extracellular space. ISIP2A has a transmembrane domain and
259 was shown to be internalized by endocytosis.⁷⁴ In contrast, FEA1 and FEA2 are secreted and the
260 mechanism for their recovery has not been identified yet.⁷⁵ There are evidences for similar
261 involvement of phyto-transferrin in many other algae, such as the halotolerant species *Dunaliella*
262 *salina*⁷⁶, *Chromera velia*⁷⁷ and *Ostreococcus tauri*.⁷⁸ Interestingly, no phyto-transferrin-based Fe uptake
263 pathway has been identified in land plants.⁷⁹ This system may have been lost upon land colonization
264 or was not present in the specific algal lineage that gave rise to land plants. Intriguingly, the expression
265 of FEA1 protein from *C. reinhardtii* is able to complement the *Arabidopsis* iron-transporter mutant *irt1*,
266 suggesting that a pathway allowing the internalization of phyto-transferrins bound to iron is likely
267 conserved in *Arabidopsis*.⁷⁹ Although algae do not produce siderophores, *P. tricornutum* is able to take
268 up Fe³⁺ chelated by siderophores of bacterial origin. Siderophore-Fe³⁺ uptake involves the ISIP1 protein
269 and its endocytosis (Fig. 1C).⁸⁰ ISIP1 is diatom specific and it is unknown whether any other algae are
270 able to take up iron via a similar mechanism. So far, there is only one report of a putative uptake of
271 the bacterial siderophore pyoverdin by land plants.⁸¹

272 Algae are also able to use a reductive pathway for Fe acquisition (Fig. 1C). It involves the Ferric
273 Reductase FRE, an homologue of FRO2 in land plants and FRE1 in yeast.^{82,83} However, some algal
274 species, such as *Ostreococcus tauri*, have very low ferric reductase activity.⁸⁴ After reduction, ferrous
275 iron should be rapidly taken up by the cell through the action of metal transporters. So far, the
276 molecular identity of such transporters is not clearly established. In *C. reinhardtii*, the transcription of
277 two Zinc and Iron regulated transport-like Proteins (ZIP) family genes is up-regulated upon Fe
278 deficiency.⁷⁵ Thus, ZIP may constitute good candidates for Fe²⁺ uptake in this species. In *P. tricornutum*,
279 ZIP and NRAMP homologues have also been identified but their subcellular localization and transport
280 abilities remain to be determined.⁸⁵ Once the Fe²⁺ transporters will be identified in algae, it would be
281 interesting to determine if they function as a complex with the ferric chelate reductase as in
282 *Arabidopsis*. In yeast, high affinity Fe uptake requires the copper-dependent ferrous iron oxidase FET3
283 that oxidized ferrous iron prior to its uptake as ferric iron by the FTR1 transporter.⁸⁶ Interestingly, FET3
284 and FTR1 proteins form a complex, which couples oxidation to uptake and prevents precipitation of
285 ferric iron after the oxidation step.⁸⁷ Similarly to yeast, a complex including the ferroxidase FOX1 and
286 the Fe³⁺ permease FTR1 has been identified in *C. reinhardtii*.⁸⁸ As for yeast FET3, copper is a cofactor
287 of FOX1 in *C. reinhardtii*, linking Cu homeostasis and Fe uptake.⁸⁹ However, contrary to yeast, copper
288 deficiency does not result in secondary Fe deficiency in *C. reinhardtii*. This may be due to the presence
289 of an alternative system to take up ferric Fe via FEA1/2 proteins, that are not present in yeast.⁸⁹
290 Homologue genes encoding FOX1 and FTR1 proteins are present in diatom genomes but their role in

291 Fe uptake has not been investigated yet.⁸⁵ In contrast, **land** plants do not seem to possess a
292 homologous Fe uptake pathway coupling Fe oxidation to high affinity uptake of ferric iron.
293 Except the involvement of a ferric reductase, the majority of the systems used in algae for iron uptake
294 are not present in land plants. These systems were probably rapidly lost upon land colonization, since
295 iron acquisition in the basal terrestrial photosynthetic organism *Marchantia polymorpha* seems to
296 mainly rely on a reductive pathway very similar to the one active in dicots.⁹⁰ Conversely, most of the
297 molecular components of strategy I and II are not present in algae, which raises intriguing questions
298 on their evolutionary origin.

299

300 **Conclusions and future work directions**

301 - Fe reduction strategy seems to be the ancestral Fe uptake mechanism of land plants and unicellular
302 algae, sharing features with the reduction strategy of other organisms such as *Marchantia polymorpha*.

303

304 - The necessity of forming a complex to optimize the process of Fe uptake when it depends on the
305 coordinated action of different proteins seems of great importance in different organisms.

306

307 - The iron uptake mechanisms in *Arabidopsis* are well described and the intracellular dynamics of the
308 proteins that form the iron-acquisition complex are starting to be uncovered, especially for the IRT1
309 iron transporter. Still, the biological importance of the formation of such a complex will have to be
310 thoroughly characterized in the future.

311

312 - In *Arabidopsis*, several ferric reductases have been identified⁹¹. They may be involved in Fe reduction
313 and subsequent acquisition in different plant tissues and organelles. Whether different iron-reducing
314 platforms exist in plants remains an open question. In the future, knowledge gained about root iron
315 uptake may be applied to study Fe mobilization in sink tissues, including the loading of iron in flowers
316 and seeds.

317

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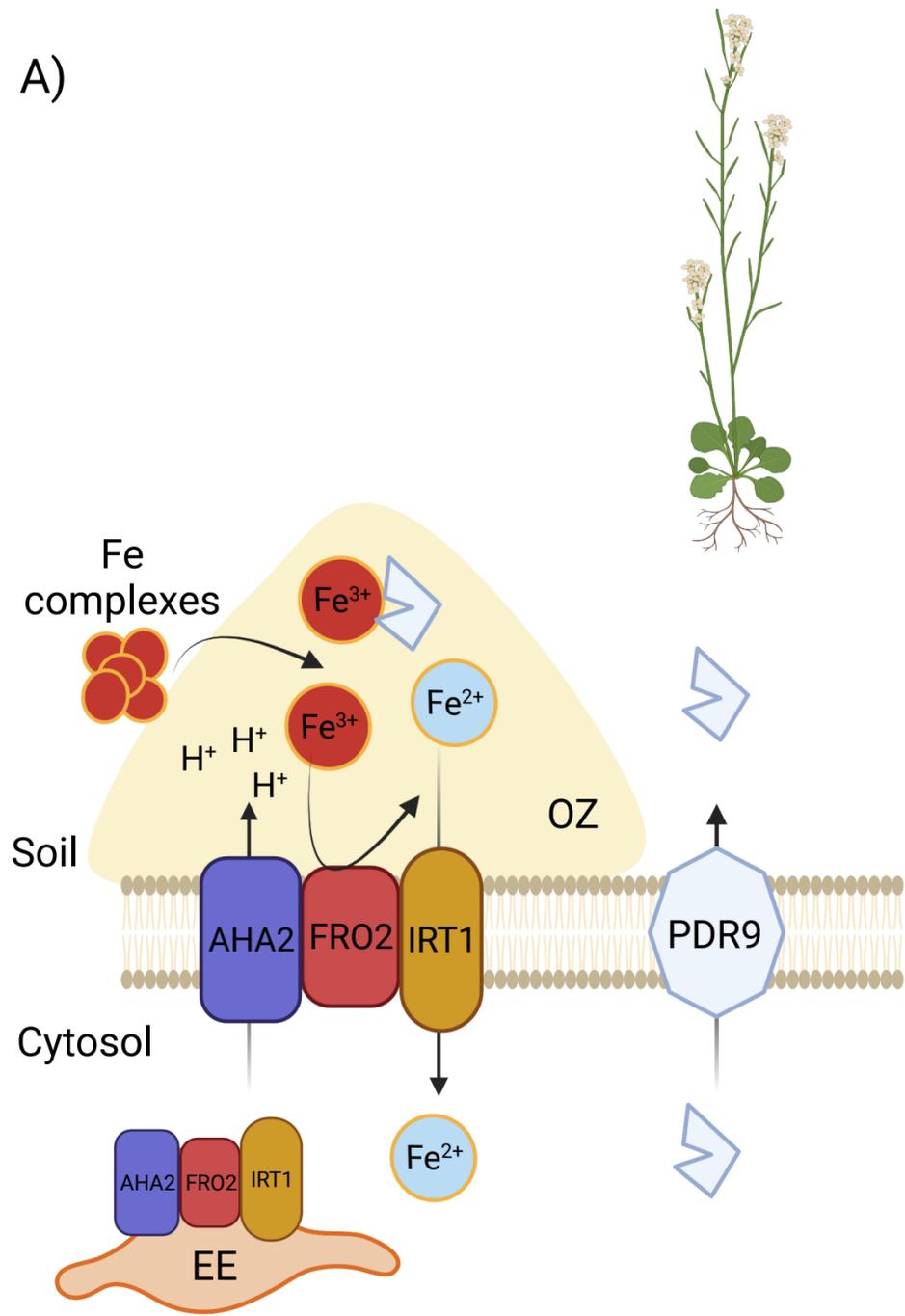
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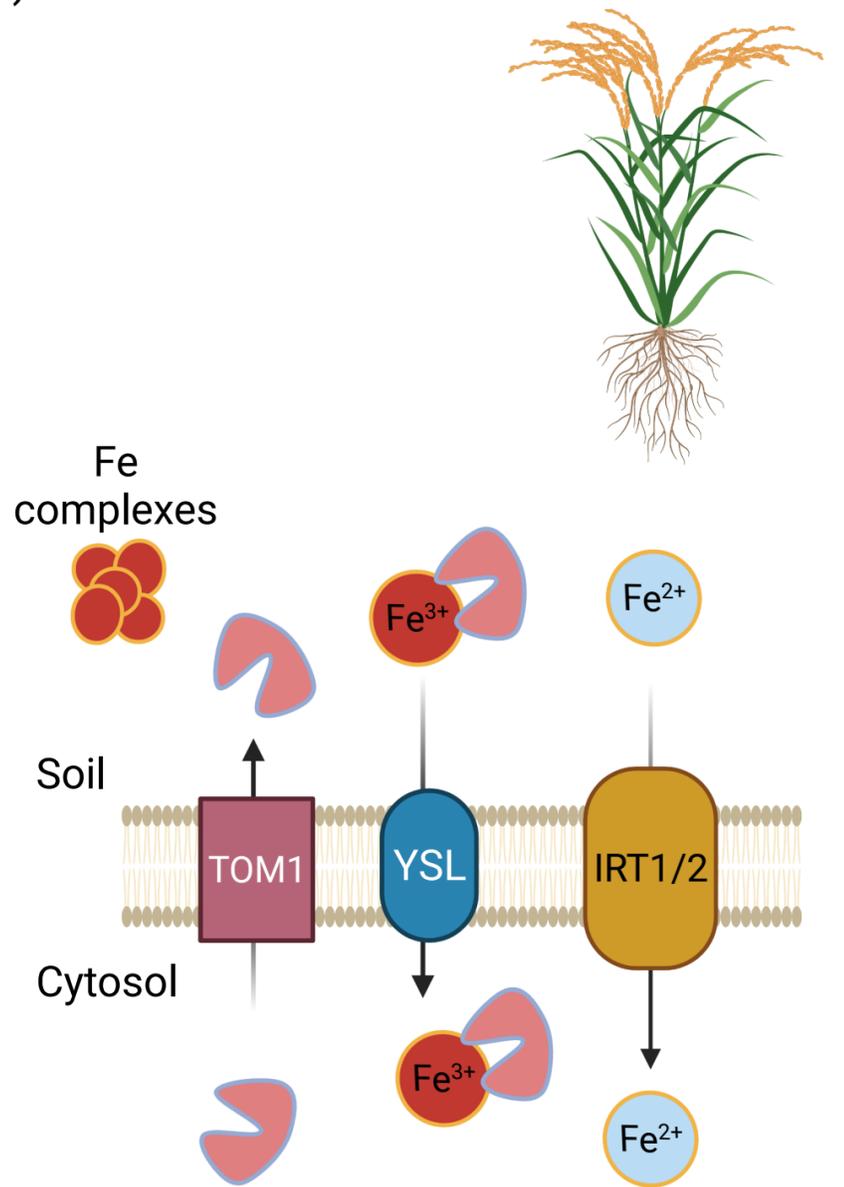
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A)



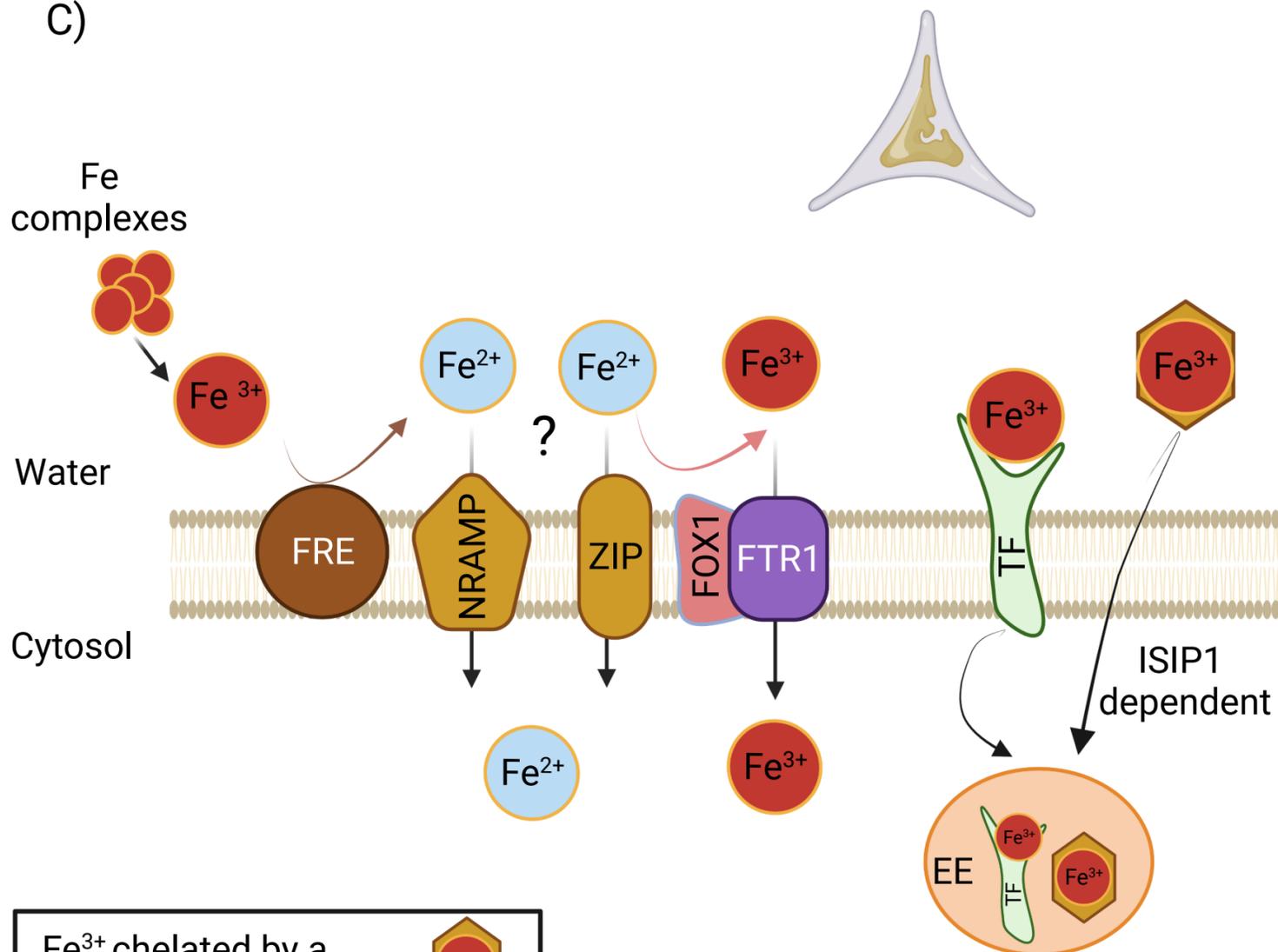
Coumarin: 

B)



Phytosiderophore: 

C)



Fe³⁺ chelated by a bacterial siderophore: 

Figure 1. The different iron acquisition strategies in photosynthetic organisms.

(A) Dicots and non-graminaceous monocots use an acidification-reduction-transport strategy for iron uptake, as exemplified here for *Arabidopsis thaliana*. The rhizosphere is acidified via proton extrusion mediated by the AHA2 proton pump, which induces the solubilization of Fe³⁺ complexes. Then, solubilized Fe³⁺ is reduced to Fe²⁺ by the FRO2 reductase and Fe²⁺ is finally transported by IRT1 inside root epidermal cells. AHA2, FRO2 and IRT1 co-localize at the outer plasma membrane domain facing the rhizosphere and in early endosomes (EE). These proteins are able to interact altogether to form an iron-acquisition complex that may optimize Fe absorption by creating a local environment with low pH and high Fe²⁺ concentration (optimal zone (OZ), represented in pale yellow). Coumarin release, mediated by PDR9 transporter, contributes to the Fe acquisition process. **(B)** Iron acquisition mechanisms in the graminaceous monocot *Oryza sativa*. TOM1 protein allows the secretion in the rhizosphere of phytosiderophores, mainly 2'-Deoxymugineic acid, that chelate Fe³⁺. Phytosiderophore-Fe³⁺ complexes are then uptaken by YSL transporters into root epidermal cells. In addition, Fe²⁺ can be directly absorbed from the soil by IRT1/2 transporters. **(C)** Multiple iron acquisition systems co-exist in different unicellular algae such as *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*. The reductive pathway for Fe acquisition involves the FRE ferric reductase. Produced Fe²⁺ may then be transported inside the cell by metal transporters from the NRAMP and ZIP families, although experimental evidences are still needed. Alternatively, Fe²⁺ can be re-oxidized by the FOX1 protein and then transported inside the cell by the FTR1 transporter. FTR1 forms a complex with FOX1 to prevent the precipitation of Fe³⁺ after the oxidation step. In some unicellular algae, transferrin-like proteins (TF) bind Fe³⁺ in the extracellular space and are then endocytosed, allowing Fe to enter in the cell. Furthermore, some algae can acquire Fe via the internalization of bacterial siderophores associated with Fe³⁺, a process involving ISIP1 protein in *P. tricornutum*. All the images presented in this figure were created with BioRender.com.