



**HAL**  
open science

## Handing off iron to the next generation: how does it get into seeds and what for?

Stéphane Mari, Christophe Bailly, Sébastien Thomine

### ► To cite this version:

Stéphane Mari, Christophe Bailly, Sébastien Thomine. Handing off iron to the next generation: how does it get into seeds and what for?. *Biochemical Journal*, 2020, 477 (1), pp.259–274. 10.1042/BCJ20190188 . hal-02445553

**HAL Id: hal-02445553**

**<https://hal.science/hal-02445553>**

Submitted on 14 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **title**

2 **Handing off iron to the next generation: how does it get into seeds and**  
3 **what for?**

4  
5 Stéphane Mari<sup>1</sup>, Christophe Bailly<sup>2</sup>, Sébastien Thomine<sup>3\*</sup>

6  
7 <sup>1</sup> BPMP, INRA, CNRS, Univ Montpellier, Montpellier SupAgro, Montpellier, France

8 <sup>2</sup> Sorbonne Université, CNRS, Laboratoire de Biologie du Développement, F-75005 Paris,  
9 France

10 <sup>3</sup> Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris-Sud,  
11 Université Paris-Saclay, 91198, Gif-sur-Yvette cedex, France

12 \*to whom correspondence should be addressed

13 [Sebastien.thomine@i2bc.paris-saclay.fr](mailto:Sebastien.thomine@i2bc.paris-saclay.fr)

14 +33 1 69 82 46 32

15

16 **Abstract**

17 To ensure the success of the new generation in annual species, the mother plant transfers a  
18 large proportion of the nutrients it has accumulated during its vegetative life to the next  
19 generation through its seeds. Iron (Fe) is required in large amounts to provide the energy and  
20 redox power to sustain seedling growth. However, free Fe is highly toxic as it leads to the  
21 generation of reactive oxygen species. Fe must therefore be tightly bound to chelating  
22 molecules to allow seed survival for long periods of time without oxidative damage.  
23 Nevertheless, when conditions are favorable, the seed Fe stores have to be readily remobilized  
24 to achieve the transition towards active photosynthesis before the seedling becomes able to  
25 take up Fe from the environment. This is likely critical for the vigor of the young plant. Seeds  
26 constitute an important dietary source of Fe, which is essential for human health.  
27 Understanding the mechanisms of Fe storage in seeds is key to improve their Fe content and  
28 availability in order to fight the Fe deficiency. Seed longevity, germination efficiency and  
29 seedling vigor are also important traits that may be affected by the chemical form under which  
30 Fe is stored. In this review, we summarize the current knowledge on seed Fe loading during  
31 development, long-term storage and remobilization upon germination. We highlight how this  
32 knowledge may help seed Fe biofortification and discuss how Fe storage may affect seed  
33 quality and germination efficiency.

34  
35

## **Introduction**

36 Seed is a very special stage in the life of a plant. It is the basis for the next generation and the  
37 survival of the species. To ensure the success of the new generation, the mother plant transfers  
38 a large proportion of the nutrients it has accumulated during its vegetative life to its seeds.  
39 Most work on seed nutrient storage focuses on carbohydrate, lipids and proteins, which  
40 provide the bulk energy, carbon and nitrogen required to initiate development. Depending on  
41 species, 30 to 90% of plant nitrogen is allocated to seeds at the end of the plant life cycle<sup>1</sup>.  
42 However, mineral stores are also very important to sustain seedling growth. Especially, iron  
43 (Fe) is required in large amount in mitochondria, which provide the energy and redox power  
44 during the initial stage of seedling development. For example, in the case of *Arabidopsis*  
45 (*Arabidopsis thaliana*), 50 to 55% of the iron (Fe) is allocated to seeds at the end of the life  
46 cycle<sup>2,3</sup>. Moreover, the nutrients need to be stored in a compact and stable form. Seed storage  
47 proteins form pseudo crystalline aggregates in protein bodies within seed embryo cells. Free  
48 Fe is highly toxic as it leads to the generation of reactive oxygen species through the Fenton  
49 reaction. In cells, Fe must therefore be tightly bound to chelating molecules to prevent the  
50 formation of ROS. This is probably even more important in seeds than in other organs, as  
51 seeds represent resistance forms designed to survive for long periods of time, often several  
52 years, and through stresses. Nevertheless, when conditions are favorable, the nutrients stored  
53 in seeds have to be readily remobilized to sustain the early development of the seedling before  
54 it acquires photosynthetic capacity and becomes able to take up nutrients from the  
55 environment. Iron is a major component of photosystem I and its availability to the young  
56 seedling is necessary to achieve the transition towards active photosynthesis. This is likely  
57 critical for the vigor of the young plant.

58 From the point of view of animals and especially human beings, seeds are a major food  
59 resource. They provide carbohydrates, proteins and fat but also micronutrients such as Fe and  
60 zinc, which are essential for human health. About 2 billion human beings suffer from Fe  
61 deficiency, which leads to asthenia, anemia and in extreme cases to death. The prevalence of  
62 Fe deficiency is highest in populations that rely mostly on a plant based diet because plants  
63 and seeds in particular do not provide sufficient Fe<sup>4</sup>. Understanding the mechanisms of Fe  
64 storage in seeds is key to improve their Fe content and availability in order to fight Fe  
65 deficiency using biofortification. From the agricultural perspective, seed longevity,  
66 germination efficiency and seedling vigor are also very important traits that breeders and  
67 farmers seek to improve. Here also, seed nutrient content and especially the content and

68 chemical form of Fe may be critical. In this review, we will summarize the current knowledge  
69 on seed Fe loading during development, long-term storage and remobilization upon  
70 germination, updating the last review on the topic <sup>5</sup>. We will highlight how this knowledge  
71 may help seed Fe biofortification, longevity, germination efficiency and stand establishment.

72

### 73 **Iron uptake and translocation to the shoots**

74 Plants take up iron using distinct strategies according to species <sup>6, 7</sup>. Most plant species use  
75 strategy I, which is based on the reduction of Fe in the rhizosphere followed by its uptake by a  
76 transporter for Fe(II). In contrast, graminaceous species, which include the major grain crops  
77 such as rice, wheat and barley, have evolved strategy II. Strategy II is based on the release of  
78 small Fe(III) chelating molecules called phytosiderophores in the rhizosphere and subsequent  
79 uptake of siderophore-Fe(III) complexes. Under Fe deficiency, strategy I plants activate the  
80 expression of the genes involved in Fe solubilization and uptake. The molecular players in  
81 this response have been identified in Arabidopsis but are essentially conserved in other  
82 species. The proton pump Arabidopsis plasma membrane H<sup>+</sup>-ATPase 2 (AHA2) drives  
83 rhizosphere acidification which is important to solubilize Fe <sup>8</sup>. The coumarin transporter  
84 Pleiotropic Drug Resistance 9 (PDR9) allows the secretion of coumarin with iron binding and  
85 reducing properties in the rhizosphere. Enzymes of the coumarin biosynthetic pathway are  
86 induced along with *PDR9* <sup>9, 10</sup>. The membrane bound Ferric chelate Reductase FRO2 reduces  
87 chelate-bound Fe(III) to Fe(II), which is taken up into root cells by the divalent metal Iron  
88 Regulated Transporter 1 (IRT1) <sup>11, 12</sup>. IRT1 is not specific for Fe(II) and drives the uptake of a  
89 range of other divalent metal cations including Zn, Mn, Co, Cd, Ni <sup>13-15</sup>. A sophisticated  
90 mechanism enables the removal of IRT1 from the plasma membrane when intracellular  
91 concentrations of non-Fe metals raise <sup>16</sup>. In addition, several genes involved in the  
92 sequestration of these metal cations are activated under Fe deficiency <sup>17</sup>. In graminaceous  
93 species such as rice, maize and barley, the components that are upregulated for Fe acquisition  
94 under Fe deficiency include the phytosiderophore efflux and Fe-siderophore influx  
95 transporters as well as the biosynthetic pathway of phytosiderophores of the mugineic acid  
96 family <sup>7</sup>. The S-adenosyl methionine cycle is activated to produce methionine, which is used  
97 by the nicotianamine synthase (NAS) to produce nicotianamine (NA) the precursor of  
98 mugineic acids. Whereas NA is also synthesized in strategy I species, mugineic acids are  
99 unique to graminaceous species. NA is converted to mugineic acid by the nicotianamine  
100 aminotransferase (NAAT) and to deoxymugineic acid by the deoxymugineic acid synthase  
101 (DMAS). MA and DMA are secreted to the rhizosphere by an efflux transporter, Transporter

102 Of Mugineic acid 1 (TOM1) in rice (*Oryza sativa*), Yellow Stripe 3 (YS3) in maize (*Zea*  
103 *mais*)<sup>18, 19</sup>. Fe-siderophore complexes are taken up by specific influx transporters, Yellow  
104 Stripe 1 (YS1) in maize and YS1-Like 15 (YSL15) in rice<sup>20-22</sup>. Despite the difference in their  
105 uptake strategies, the control of Fe deficiency responses involves a similar network of  
106 negatively and positively regulating bHLH (basic Helix Loop Helix) in graminaceous plants  
107 and the other species<sup>23, 24</sup>. In addition, Hemerythrin Domain containing RING proteins that  
108 act as negative regulators of Fe deficiency responses by mediating the Fe dependent  
109 degradation of specific bHLH, are also conserved among strategy I and strategy II plants<sup>25-27</sup>.  
110 To be translocated to the shoots, Fe has to go through the endodermis cell layer and to be  
111 released into the xylem sap together with ligands that maintain it in soluble form. Recently,  
112 suberin deposition around the endodermis has been shown to provide a control point for Fe  
113 translocation. Under Fe deficiency, the suberin layer is degraded allowing the transfer of Fe  
114 from the root cortex to the central cylinder where it can be loaded into the xylem for  
115 translocation to the aerial parts<sup>28, 29</sup>. Based on its expression pattern and plasma membrane  
116 localization, the Fe efflux transporter Ferroportin (FPN1)/Iron REGulated 1 (IREG1) has been  
117 proposed to mediate Fe loading in the xylem in Arabidopsis<sup>30</sup>. The MATE family citrate  
118 efflux transporters, Ferric Reductase Deficient 3 (FRD3) in Arabidopsis and FRD3- Like  
119 1(FRD1) in rice, play an important role in the translocation of Fe and other metals, by  
120 loading citrate which chelates Fe and prevent its precipitation in the xylem sap<sup>31-35</sup>.

121

## 122 **Remobilization from other organs to the seeds**

123 At the vegetative stage, most of the iron translocated to aerial parts is used for photosynthesis  
124 in chloroplasts in mesophyll cells. Photosystem I and Ferredoxin, which are very abundant in  
125 chloroplasts, contain numerous FeS clusters. This assumption based on biochemical needs is  
126 supported by cell fractionation experiments and Fe imaging in leaves<sup>36-38</sup>. Upon transition to  
127 the reproductive stage, a large part of the Fe present in vegetative tissues is transferred to the  
128 seeds. In Arabidopsis, 50 to 55% of total plant Fe ends up in the seeds at the end of the life  
129 cycle (the Iron Harvest Index, **Figure 1**)<sup>2, 3</sup>. A recent study based on Fe isotope labeling  
130 indicated that most of the Fe stored in seeds originates from vegetative tissue, implying a  
131 major remobilization of Fe<sup>2</sup>.

132 Prior to its movement from the vegetative tissues to the seeds, Fe has to be made available in  
133 the senescing tissues. Autophagy was proposed to play a key role in nutrient remobilization  
134 from vegetative tissues to seeds<sup>39</sup>. This was based on the finding that autophagy is involved  
135 in nitrogen remobilization to seeds<sup>40</sup>. Recently, the finding that a mutation in *Autophagy 5*

136 (*ATG5*), which strongly impairs autophagy, decreases the proportion of Fe allocated to seeds  
137 from 50-55% to 15-20% highlighted the importance of autophagy for Fe remobilization <sup>2</sup>.  
138 Similar results were obtained for other micronutrients such as Zn and Mn. The role of  
139 autophagy is thus clearly not limited to Fe. Recently, several pathways have been implicated  
140 in the degradation of chloroplasts, which contain most of the cellular Fe, during leaf  
141 senescence <sup>41</sup>. They include (i) the senescence-associated vacuoles (SAV) that are important  
142 for the degradation of stromal proteins such as RuBisCo and glutamine synthase, (ii)  
143 chlorophagy in which whole chloroplast are encapsulated in autophagosomes <sup>42</sup> (iii) a  
144 vesicular pathway depending on the ATG8 Interacting protein (ATI) which degrades envelope  
145 and thylakoid membranes in addition to stromal proteins <sup>43</sup> and (iv) CV-Containing vesicles  
146 (CCVs) that contain the CV protein (Chloroplast Vesiculation) responsible for chloroplast  
147 destabilization <sup>44</sup>. In contrast to the others, the CCV pathway does not require autophagy. It  
148 will be interesting to determine the respective importance of these pathways for Fe  
149 remobilization during vegetative organ senescence, as they could allow to target some  
150 nutrients more specifically than autophagy.

151 In addition to autophagy, the timing of senescence is also an important factor controlling  
152 remobilization. If senescence occurs too fast, the time window for remobilization is reduced  
153 and less nutrients are allocated to seeds. Hence, NAC transcription factors, which control the  
154 onset of senescence, also play a major role in the control of nutrient remobilization from  
155 leaves to seeds <sup>45-47</sup>. More work will be required to understand how the interplay between  
156 autophagy and senescence controls Fe allocation to seeds in different species.

157 After it has been made available in vegetative tissue, Fe has to be transported to developing  
158 seeds through the phloem. Many lines of evidence have pointed to a key role of NA in this  
159 process. Complete loss of NA synthesis leads to sterility <sup>48-50</sup>. This has initially prevented a  
160 direct analysis of the role of NA in Fe transport to seeds, as the mutants did not set seeds. The  
161 first evidence that NA is important for Fe transport to the seeds came from the study of  
162 mutants impaired in *YSL-like* genes (*YSL*). The strong expression of *OsYSL2* in phloem tissues  
163 of rice aerial parts and developing seeds and the ability of this transporter to take up NA-Fe  
164 and NA-Mn complexes when expressed in *Xenopus* oocytes provided the first indication that  
165 NA is important for Fe transport through the phloem to the seeds <sup>51</sup>. Analysis of rice lines in  
166 which *OsYSL2* expression was silenced further supported this hypothesis <sup>52</sup>. In these lines, Fe  
167 transport to the shoots and the seeds was strongly decreased. Conversely, increased *OsYSL2*  
168 expression under a phloem specific promoter led to strong increase in seed endosperm Fe  
169 concentration <sup>52</sup>. More recently, another YSL transporter from rice, *OsYSL9*, was implicated

170 in Fe delivery to developing rice grain. *OsYSL9* expression is upregulated by Fe deficiency in  
171 roots but down regulated in leaves under the same conditions <sup>53</sup>. The role of YSL in metal  
172 transport to seeds is not restricted to rice. Similar conclusions were obtained from the analysis  
173 of Arabidopsis knockout mutants targeting *AtYSL1* gene or double mutants affecting *AtYSL1*  
174 and *AtYSL3* <sup>54-56</sup>. Like *OsYSL2*, *AtYSL1* is expressed in phloem tissues of leaves as well as in  
175 developing siliques. Similar to *OsYSL9*, it is down regulated under Fe deficiency. Loss of  
176 function mutants in *AtYSL1* accumulated higher levels of NA in shoots but strongly decreased  
177 NA and Fe concentrations in mature seeds <sup>55</sup>. Combining a mutation in *AtYSL1* with a  
178 mutation in another Arabidopsis YSL strongly expressed in phloem, *AtYSL3*, leads to more  
179 severe phenotypes: the *ysl1ysl3* mutants display symptoms of strong Fe deficiency with  
180 interveinal chlorosis as well as decreased fertility. Like *ysl1*, they also accumulate lower  
181 concentrations of Fe in their seeds when provided with a high Fe concentration in the  
182 medium, which alleviates the defect in fertility and up-regulate *YSL1* expression <sup>55, 56</sup>. Further  
183 inflorescence grafting experiments showed that expression of *AtYSL1* and *AtYSL3* in the  
184 rosette was sufficient to restore fertility but not seed Fe content <sup>54</sup>. Furthermore, the  
185 identification of a quadruple mutant combining knockout in 3 out of the 4 genes encoding  
186 NAS in Arabidopsis and a knockdown in the fourth one allowed to obtain fertile plants with  
187 drastically decreased levels of NA <sup>48</sup>. In this quadruple *nas* mutant, Fe and NA levels are  
188 lower in seeds, similar to the phenotype observed in *ysl1*. Interestingly, in NA biosynthesis  
189 mutants, Fe accumulates in phloem cells indicating that NA is required to retrieve Fe from the  
190 phloem and provide it to sink tissues, such as seeds <sup>57</sup>. This role may be shared between NA  
191 and mugineic acids in graminaceous plants. On the other hand, all YSL transporters  
192 characterized so far using expression in yeast or in *Xenopus* oocytes mediate the influx of  
193 NA-Fe or MA-Fe complexes into cells. The finding that NA and YSL are important for  
194 phloem transport of Fe does not imply that Fe is transported as NA-Fe complex in the phloem.  
195 Another important player in Fe transport from vegetative organs to the seeds is the  
196 oligopeptide transporter OligoPeptide Transporter 3 (OPT3). Full loss of function of *OPT3*  
197 leads to embryo lethality suggesting that the substrate of OPT3 is an essential cellular  
198 metabolite <sup>58</sup>. Even though the closest homologue of OPT3, BjGT1, is a glutathione  
199 transporter <sup>59</sup>, transport assays in yeast failed to show OPT3 ability to transport GSH <sup>60</sup>.  
200 Instead, expression of *OPT3* could complement the yeast *fet3fet4* mutant impaired in Fe  
201 uptake, suggesting that it transports Fe complexes. The exact substrate of OPT3 remains to be  
202 identified. The identification of a weak allele of *opt3*, *opt3-2* allowed investigating its  
203 function at the reproductive stage <sup>61</sup>. In *opt3-2* mutants, Fe concentration is increased in

204 vegetative organs but decreased in seeds, indicating a role in Fe transfer from vegetative  
205 organs to seeds. More recently, OPT3 was shown to be targeted to the plasma membrane and  
206 mainly expressed in phloem cells<sup>60, 62</sup>. In *opt3-2* mutants, Fe concentration was strongly  
207 increased in the xylem sap and decreased in the phloem sap, pointing to role of OPT3 in Fe  
208 transfer from the xylem to the phloem<sup>60</sup>. Combined with its expression in phloem cells, this  
209 suggests that OPT3 is involved in Fe loading into the phloem. Hence, both OPT3 and NA  
210 play critical roles in long distance transport of Fe in the phloem to sink organs and especially  
211 seeds: OPT3 would be necessary for loading Fe into the phloem, while NA would be required  
212 for Fe retrieval from the phloem<sup>57, 60</sup>. Interestingly, OPT3 is also important for Fe deficiency  
213 signaling and *opt3-2* up-regulated Fe-deficiency responsive gene expression even under Fe  
214 sufficient conditions<sup>60, 62</sup>. In rice, OsOPT7, a homologue of OPT3, was characterized<sup>63</sup>.  
215 OsOPT7 could not transport GSH or Fe when expressed in *Xenopus* oocytes and yeast. Like  
216 *AtOPT3*, *OsOPT7* is induced under Fe deficiency but its expression is more widespread than  
217 that of *OPT3*<sup>61, 63</sup>. Moreover, the phenotype of *opt7* mutant does not resemble that of *opt3-2*.  
218 Whether graminaceous plant genomes carry orthologues of OPT3 remains to be determined.

219

### 220 **Iron loading into seed tissues**

221 There is no continuity between the vasculature of the mother plant and the pro-vasculature of  
222 the embryo. Therefore, Fe, as other nutrients, has to be released from the phloem and re  
223 absorbed by the embryo. In Arabidopsis and other dicotyledonous plants, nutrients are  
224 released at the level of the chalazae and the nucellus into the embryo sac fluid from which the  
225 embryo takes up nutrients (Figure 2). Accordingly, *AtYSL1* expression is detected in the  
226 funiculus and the chalazal endosperm<sup>55</sup>. Analyses of Fe speciation in the embryo sac fluid of  
227 developing pea seeds revealed that Fe is present as ferric Fe bound to citrate and malate in this  
228 extracellular compartment<sup>64</sup>. In agreement with this finding, the citrate efflux transporter  
229 *FRD3* is expressed in the peripheral cell layer of the embryo and the cell layer of the tegument  
230 facing the embryo sac during seed development (Figure 2)<sup>34</sup>. This suggests that *FRD3* secretes  
231 citrate in the embryo sac to maintain Fe solubility and availability for uptake by the embryo.  
232 In dicotyledonous plants, Fe is taken up as ferrous Fe. This implies that Fe must be reduced  
233 prior to uptake by the embryo. However, genetic analyses in Arabidopsis failed to identify a  
234 membrane bound ferric chelate reductase that is important for Fe acquisition by the embryo  
235 among *FRO2* homologues. Instead, further analysis of the embryo sac fluid in pea showed the  
236 presence of a high concentration of ascorbate, sufficient to reduce Fe(III) to Fe(II) prior to its  
237 uptake by the embryo<sup>64</sup>. Consistently, in Arabidopsis *vtc* (*vitamin c*) mutants deficient in



238 ascorbate biosynthesis, the seed Fe content is decreased. The speciation of Fe in the embryo  
239 sac is thus similar to that encountered in other extracellular compartments of the plant, such as  
240 the xylem sap. In contrast, it is striking that ascorbate is used for Fe reduction, whereas  
241 membrane bound ferric chelate reductases are used for uptake in roots and leaves as well as in  
242 intracellular organelles such as mitochondria and plastids<sup>11, 65, 66</sup>. The transporters responsible  
243 for secreting ascorbate and Fe in the embryo sac remain to be identified. In the case of Zn, the  
244 Heavy Metal pumping P-type ATPases HMA2 and HMA4 release Zn from the mother tissues  
245 for subsequent uptake by the embryo<sup>67</sup>. In the case of Fe, the plasma membrane Fe efflux  
246 transporter IREG1/FPN1 may be involved in this process but so far no defect in Fe supply to  
247 the embryo has been reported for *ireg1/fpn1* mutant<sup>30</sup>.

248 In wheat and barley, the nutrients, including Fe, are provided to the grain by a single vascular  
249 strand along the ventral crease<sup>68</sup>. Fe is mostly provided by the phloem and moves through  
250 several specialized cell layers: the crease vascular parenchyma, the pigment strand and the  
251 nucellar projection. Fe ends up in the transfer cells that are facing the embryo. From there, Fe  
252 has to be released in the extracellular space separating the mother plant from the embryo  
253 (Figure 3). The transfer cells, as the modified aleurone cells facing them on the side of the  
254 embryo, have highly invaginated plasma membranes favoring nutrient release and  
255 reabsorption<sup>68</sup>. Fe accumulation and speciation in these structures show marked contrasts in  
256 the mature wheat grain. Fe is highly concentrated in the nucellar projection and co-localizes  
257 with sulfur. Fe accumulates to a lesser extent in the modified aleurone, from where it is  
258 probably distributed to the other aleurone cells, the embryo and the endosperm<sup>69</sup>. X-ray  
259 absorption spectra indicate that in the nucellar projection, Fe is mostly associated with NA,  
260 whereas in the modified aleurone it is associated with phytate<sup>69</sup>. In agreement with the  
261 speciation, barley homologues of AtVIT1, which drives Fe influx into the vacuole where  
262 phytate is localized, are strongly expressed in the aleurone<sup>68, 70-72</sup>. In contrast, the genes  
263 encoding NAAT, NAS and YSL, that favor Fe mobility, are expressed at high levels in  
264 transfer cells<sup>68, 73</sup> (Figure 3). In the future, it will be important to determine the specific  
265 expression pattern and the role of each of the genes involved in Fe transport in this complex  
266 structure. This should allow the identification of the key transporters that release Fe to the  
267 extracellular space from the transfer cells as well as those responsible for taking it up into the  
268 embryo in the modified aleurone cells.

269

270 **Iron storage in seeds**

271 The localization of Fe in seeds differs according to species and seed developmental stage. The  
272 localization and subcellular localization are strongly associated with Fe speciation, i.e. the  
273 nature of the ligand that binds Fe and determines its bioavailability<sup>74</sup>. For example, Fe phytate  
274 complexes that are stored in vacuoles are notoriously poorly bioavailable<sup>75</sup>. In contrast,  
275 ferritin Fe stored in plastids constitutes a highly bioavailable source of Fe<sup>76</sup>.

276 Iron and other metals are not distributed evenly in seed tissues. In contrast, Fe distribution  
277 follows striking patterns. For example, in *Arabidopsis* mature embryo, Fe is highly  
278 concentrated around the vascular tissues<sup>71, 77-81</sup>. Interestingly, the pattern of Fe distribution is  
279 distinct from that of other metals: Mn is concentrated in the sub-epidermal cell layers of  
280 *Arabidopsis* cotyledons in mature seeds while Zn is evenly distributed in *Arabidopsis* embryo  
281<sup>71, 77, 78, 81</sup>. The patterns of metal localization were initially discovered using synchrotron X-ray  
282 Fluorescence imaging of intact seeds and were then confirmed and refined using additional  
283 approaches. The use of Perls staining intensified by Diaminobenzidine (Perls DAB) allowed  
284 identification of the precise cell type accumulating Fe as the proto endodermis of the embryo  
285<sup>80</sup>. The use of micro Particle Induced X-ray Emission ( $\mu$ PIXE) allowed quantification of the  
286 pattern: even though it is concentrated in proto-endodermal cells, Fe is present in other cells,  
287 albeit at much lower concentrations, and the Fe concentrated around pro-vascular tissues  
288 accounts for 50% of total seed Fe<sup>81</sup>. Finally, the use of Electron Dispersive X-ray imaging  
289 coupled to Transmission Electron Microscopy (TEM EDX) provided higher spatial resolution  
290 allowing the determination of the subcellular localization of Fe in the globoids of vacuoles of  
291 proto-endodermal cells (Figure 4)<sup>79, 82</sup>. This subcellular localization is in agreement with the  
292 finding that mutations in *AtVIT1* (Vacuolar Iron Transporter 1) disrupt the pattern of Fe  
293 distribution<sup>71, 79</sup>. In *vit1* mutants, Fe is not concentrated around vascular tissues but instead  
294 co-localizes with Mn in sub-epidermal cells<sup>71, 79</sup>. Nevertheless, Fe is still localized in  
295 vacuoles in *vit1* mutants<sup>79</sup>. Recently, the vacuolar Mn transporter *AtMTP8* (Metal Tolerance  
296 Protein 8) was shown to be responsible for concentrating Mn in sub-epidermal cells in wild  
297 type seeds and Fe localization in these cells in the *vit1* mutant background<sup>77, 78</sup>. In *mtp8*  
298 knockout mutant seeds, Mn is concentrated around the vascular tissues together with Fe. In a  
299 double mutant combining *vit1* and *mtp8* mutations, Mn and Fe are evenly distributed in  
300 *Arabidopsis* embryo<sup>78</sup>. Therefore, by creating strong sinks for Fe or Mn, vacuolar metal  
301 transporters determine the pattern of metal distribution in mature *Arabidopsis* embryo. The  
302 phenotype of *vit1* mutant, which survives poorly when germinated under Fe-deficient  
303 conditions, indicates that adequate tissue localization in the embryo is crucial<sup>71, 79</sup>. Little  
304 information is available on how the Fe distribution pattern is generated during embryo

305 development. Consistent with a strong expression of VIT1 during seed development, Fe  
306 patterning is already apparent at the torpedo stage at the center of the cotyledons where the  
307 provascular tissue will differentiate<sup>71, 80</sup>. However, in *Brassica napus*, high resolution Perls  
308 DAB imaging detects Fe in the nuclei of all embryo cells at the torpedo stage<sup>83</sup>. Further  
309 during development, at the bent cotyledon stage, Fe is detected in cytoplasmic vesicles and  
310 finally ends up in the vacuoles of the cells surrounding the vasculature in mature embryo<sup>83</sup>.  
311 The observation of Fe in the nucleus at the torpedo stage in *B. napus* is consistent with the  
312 detection of high Fe concentrations in the nucleus of embryo cells in developing pea (*Pisum*  
313 *sativum*) seeds<sup>84</sup>. Therefore, Fe subcellular localization evolves during embryo development.  
314 These changes in localization are most likely paralleled by changes in speciation and therefore  
315 of Fe bioavailability. The analysis of Fe bioavailability in pea seeds at different  
316 developmental stages show that Fe bioavailability in immature peas is higher than in mature  
317 pea seeds<sup>85</sup>.  
318 Whether the pattern of Fe distribution in the vacuoles of cells surrounding the vasculature  
319 described in Arabidopsis is conserved among angiosperm has been investigated. In beans  
320 (*Phaseolus vulgaris*), Fe stores also concentrates around vascular tissues<sup>86</sup>. Recently, this  
321 observation was extended to Brassicales and even Rosids, which represent a wide group  
322 including a third of the angiosperms<sup>87, 88</sup>. However, in many cases the number of cell layers  
323 that accumulate Fe is increased, to 2, the endodermis and one cortical cell layer in Brassicales,  
324 or even more cortical cell layers in other Rosids, compared to endodermis only in  
325 Arabidopsis<sup>83, 87, 88</sup>. While screening representatives of the different groups of angiosperm,  
326 interesting exceptions were identified in *Chenopodium quinoa* and *Carica papaya*<sup>87, 88</sup>. There  
327 are certainly more diverse patterns to be discovered.  
328 In grains, the seeds of graminaceous species, the distribution of Fe is very different from that  
329 observed in Brassicales. In grains, in contrast to Arabidopsis, the embryo represents only a  
330 small volume of the seed, while the major part is constituted by the starchy endosperm. The  
331 endosperm is surrounded by the so called aleurone cell layer. During germination, the  
332 aleurone layer is activated by gibberellins, releases enzymes that digest the carbohydrates  
333 stored in the endosperm and undergoes programmed cell death<sup>89, 90</sup>. The embryo is equipped  
334 with an absorptive structure called the scutellum, somewhat equivalent to human placenta,  
335 which take up the nutrients from the endosperm. In grains, a large part of the Fe is  
336 concentrated in the aleurone layer and another pool is found in the embryo<sup>69, 91, 92</sup>(Figure 3).  
337 In wheat (*Triticum durum*), the highest concentrations of Fe are found in the aleurone layer  
338 and in the scutellum<sup>92</sup>. Separate measurements of wheat flour and bran indicated that almost

339 60% of grain Fe is in the bran which includes the aleurone layer and therefore represents the  
340 major Fe pool in grains<sup>93</sup>. Within the aleurone layer, Fe is concentrated together with  
341 phosphorus and other minerals in globoids inside the protein storage vacuole, similar to the  
342 subcellular localization observed in Arabidopsis<sup>72</sup>. In situ X-ray absorption spectroscopy  
343 analyses have indicated that in the aleurone layer, most Fe is bound to phytate, either as Fe(II)  
344 or Fe(III) in good agreement with its subcellular localization<sup>69, 92</sup>. Another pool is bound to  
345 citrate, which may be involved in the transport from modified aleurone cells present in the  
346 crease to aleurone cells<sup>69</sup>. In rice, Fe accumulates in the aleurone layer together with phytate  
347 and in the scutellum, as observed in wheat<sup>91, 94</sup>. The exact speciation of Fe in the embryo and  
348 in the endosperm has not yet been determined in these tissues that contain lower Fe  
349 concentration, probably because of limitations in the sensitivity of X-ray absorption  
350 spectroscopy. Based on co-occurrence of elements, it has been proposed that Fe is bound to  
351 phosphate (possibly as phytate) in the scutellum in wheat and to other ligands in the other  
352 parts of the embryo and in the endosperm<sup>69, 92</sup>. These ligands could include proteins or  
353 smaller molecules such as NA, which is present in grains as in seeds of non graminaceous  
354 species<sup>55, 95</sup>. In wheat flour which corresponds to the starchy endosperm, NA is the main  
355 ligand for Fe<sup>96</sup>.

356 Little is known on the mechanisms that control the pattern of Fe localization in grains. A  
357 developmental analysis showed that throughout rice grain development Fe co-localizes with  
358 phosphorus in the aleurone cell layer, indicating that Fe is most likely bound to phytate as  
359 soon as it is stored<sup>91</sup>. Mutations in OsVIT1, OsVIT2 and Mitochondrial Iron Transporter  
360 (MIT) have been shown to perturb iron localization within the embryo<sup>97, 98</sup>. OsYSL9 is  
361 strongly expressed in the scutellum and participates to Fe storage in the embryo<sup>53</sup>. The  
362 phyto siderophore efflux transporter TOM2 is expressed in the dorsal vascular bundle,  
363 epithelium and the scutellum of the embryo and may also participate in the distribution of Fe  
364<sup>19</sup>. Silencing TOM2 gene expression did not alter seed total Fe content. Analysis of Fe  
365 distribution using sXRF, Perls staining or seed dissection could provide more insights in its  
366 function in developing rice grains.

367

### 368 **Biofortification**

369 The abundance, the localization and the speciation of Fe are equally important parameters to  
370 be taken into account in attempts to increase Fe content and bioavailability in grains, which  
371 are two of the major targets of biofortification projects. Having the major Fe pool stored as Fe  
372 phytate in the aleurone layer is probably the worst combination for human nutrition, as

373 phytate has extremely high affinity for metals, which drastically limits Fe absorption in the  
374 intestine <sup>75</sup>. Moreover, the aleurone layer is most of the time discarded during grain  
375 processing, such as polishing for rice or preparation of white flour for wheat <sup>93, 96, 99</sup>. Hence,  
376 biofortification strategies using targeted molecular approaches have aimed at changing both  
377 the localization and the speciation of Fe in grains. Several reviews have been recently  
378 published on the attempts made to biofortify wheat and rice grain<sup>99-101</sup>. This review will  
379 therefore not extensively cover the topic of biofortification and rather just highlight a few  
380 examples. The initial attempt to biofortify rice grains through biotechnology used the gene  
381 encoding ferritin, which provides Fe under a highly bioavailable form, driven by an  
382 endosperm specific promoter <sup>102</sup>. Although the work provided a critical proof of concept, the  
383 increase in Fe content was modest and variable <sup>103</sup>. Probably the most efficient strategy for  
384 biofortification by manipulating a single gene has been the activation or the over expression  
385 of NA synthase in rice grains <sup>95</sup>. This resulted not only in a strong increase in grain Fe  
386 concentration but also in Fe bioavailability, as NA appears to favor Fe intestinal absorption <sup>95</sup>.  
387 More recently, expression of a Fe vacuolar transporter TaVIT2 in wheat endosperm was also  
388 shown to be a relevant approach to increase Fe content and bioavailability <sup>70</sup>. Probably, the  
389 expression of TaVIT2 in the endosperm vacuoles creates a strong Fe sink during  
390 development. Subsequently, Fe becomes available when endosperm cells die and lose their  
391 internal structure. Current efforts use pyramiding of several constructs to combine the benefits  
392 of multiple transgenes. This way, by combining the expression of transporters that limit  
393 vacuolar storage with elevated ferritin and NA synthesis, an increase in Fe concentration in  
394 grains by up to 6-fold has been obtained <sup>104</sup>.

395

### 396 **A role for iron in the regulation of seed germination?**

397 Seed germination starts with water uptake by the dry seed and ends with radicle protrusion  
398 and initiation of cell division <sup>105</sup>. This is the critical phase of plant emergence because it is  
399 tightly regulated by water availability, temperature, oxygen, and light conditions. Germination  
400 is also controlled by endogenous factors such as the plant hormones abscisic acid (ABA),  
401 gibberellins (GA) and ethylene that play a major role in regulating early seed germination.  
402 These endogenous signals regulate germination through the process of dormancy, which is an  
403 endogenous block to the completion of germination of a mature seed <sup>106</sup>. Germination is  
404 followed by seedling growth during which mobilization of reserves and nutrient stored within  
405 the seed sustain active cell metabolism till the acquisition of autotrophy. Thus, stand

406 establishment, the first critical component of crop yield, results from the successful and fast  
407 completion of both germination and early seedling growth.

408 Although the role of iron in post-germinative events is quite well documented (see below) its  
409 possible involvement in the regulation of germination, *i.e.* the sum of molecular events that  
410 allow radicle protrusion, is rather unknown. The effect of iron on seed germination has mostly  
411 been documented in the context of soil toxicity, since high Fe concentrations are toxic and  
412 inhibit germination<sup>107, 108</sup>, but this is a common feature to many metals. Increase in phytate-  
413 degrading enzyme activity has been reported to occur during seed germination, with a  
414 concomitant decline in phytate<sup>109-111</sup> thus increasing iron bioavailability<sup>109</sup>. Müller et al.  
415 (2009) showed that hydroxyl radicals were likely to play a role in cell wall loosening during  
416 radicle elongation and weakening of the endosperm of cress seeds<sup>112</sup>. Although Fenton  
417 reaction is a natural candidate mechanism for explaining hydroxyl radical production they did  
418 not provide any evidence about its possible involvement *in vivo*. Thus, whether iron  
419 availability participates to the numerous biochemical and molecular processes involved in the  
420 regulation of radicle protrusion is actually not known.

421 Murgia and Morandini (2017) demonstrated that, when seeds developed on the mother plant  
422 under Fe deficiency, they were more dormant at harvest, thus suggesting that seed Fe  
423 availability can regulate *sensu stricto* germination<sup>113</sup>. It is highly likely that Fe could regulate  
424 germination by controlling ROS homeostasis, through its involvement in the Fenton reaction.  
425 ROS indeed have been proposed to be key players in seed germination and dormancy<sup>114-116</sup>.  
426 Seed dormancy alleviation requires controlled ROS generation<sup>117</sup> but excessive levels of  
427 ROS trigger oxidative stress and prevent germination. This is occurring when seeds are  
428 germinated in unappropriated environmental conditions or when they have aged<sup>115</sup>. With  
429 regards to the relationship between Fe and ROS metabolism, we can thus hypothesize that Fe  
430 homeostasis, as controlled by sequestration and release by vacuoles, is likely to participate in  
431 the ability of seeds to germinate and to play a role in the regulation of dormancy, by buffering  
432 ROS homeostasis. However whether Fe speciation (and localization) is important for seed  
433 longevity and control of dormancy and germination remains to be addressed.

434

### 435 **Iron remobilization after germination**

436 Besides human nutrition, the main role of seed Fe stores is to sustain the early development of  
437 seedlings after germination. In dicots in which most of the Fe is probably stored as Fe phytate  
438 in vacuoles, retrieving Fe stores likely requires first to break down the phytate, then to  
439 transport Fe out of the vacuole and out of the cells. In Arabidopsis, Perls DAB staining

440 allowed monitoring the fate of Fe stores after germination: the high Fe concentration around  
441 vascular tissues observed in dry seeds disappeared within 4 days, indicating that Fe was  
442 rapidly redistributed the growing organs of the seedling <sup>79, 80</sup>. In Arabidopsis, this process  
443 requires two redundant vacuolar transporters AtNRAMP3 and AtNRAMP4 (Natural  
444 Resistance Associated Macrophage Protein) <sup>82</sup>. AtNRAMP3 and AtNRAMP4 are highly  
445 expressed after radicle protrusion. In loss of AtNRAMP3 and AtNRAMP4 function mutants,  
446 seedling growth is arrested before the onset of photosynthesis <sup>82</sup>. In the *nramp3nramp4*  
447 double mutant, Fe remains blocked inside the endodermal vacuoles after germination <sup>79, 80, 82</sup>.  
448 The phenotype of *nramp3nramp4* is partially suppressed by mutations in AtVIT1 by  
449 redirecting Fe storage in vacuoles of cortical cells <sup>79</sup>. The phenotype of *nramp3nramp4* is also  
450 rescued by Fe supplementation. However, even in the presence of Fe in the medium, the  
451 mutant activates Fe deficiency responses <sup>118</sup>. Interestingly, chloroplast functions and  
452 especially Fe-requiring plastidial enzymes are repressed in the mutant while mitochondrial  
453 function is maintained <sup>118</sup>. This suggests that Fe is prioritized to mitochondria or that these  
454 organelles rely on an independent pool of Fe. AtNRAMP3 and AtNRAMP4 encode divalent  
455 cation transporters. As Fe is most likely stored as Fe(III) in the vacuole, it means that Fe  
456 reduction is probably required prior to Fe export by AtNRAMP3 and AtNRAMP4. However,  
457 the Fe reduction system active in vacuoles of germinating cells remains to be identified. The  
458 phytase allowing Fe release prior to its reduction and export also remains to be identified. The  
459 importance of such activity is highlighted by the finding that expression of the bacterial  
460 phytase US417 or mutation in the phytate biosynthetic enzyme IPK1 (Inositol Phosphate  
461 Kinase 1) in Arabidopsis significantly accelerate Fe remobilization <sup>119</sup>. After its export from  
462 the vacuole, Fe needs to be exported from storage cells (the endodermis in Arabidopsis).  
463 However, the transporters involved in Fe efflux from cells during germination have not been  
464 identified. One candidate could be IREG1/FPN1, the plant homologue of the transporter  
465 involved in the release of Fe into the blood flow from intestine epithelial cells <sup>30</sup>. Once it is  
466 exported from the cells, Fe needs to remain in a soluble form to diffuse to other cells. The  
467 citrate efflux transporter FRD3 is expressed in germinating seeds and could be involved in the  
468 release of citrate to form soluble Fe complexes in the apoplast. This hypothesis is supported  
469 by the finding that *frd3* mutants are chlorotic and exhibit slow root growth after germination  
470 <sup>34</sup>. This phenotype can be rescued by supplementation of Fe or citrate in the cytosol.  
471 In relation with their distinct organization, Fe remobilization mechanisms in seeds of  
472 graminaceous species are likely very different. In grains, most of the Fe is in the aleurone  
473 layer, outside of the embryo. During germination, under the positive control of gibberellins,

474 aleurone cells secrete amylases that digest the starch in the endosperm and eventually undergo  
475 programmed cell death (PCD)<sup>89</sup>. Membranes are destroyed in this process and it is likely that  
476 the content of globoids including Fe bound to phytate is released in the endosperm. Iron  
477 efflux from vacuoles is probably not a necessary step for mobilization. Accordingly, no  
478 orthologues of AtNRAMP3 or AtNRAMP4 have been reported in graminaceous plants. In the  
479 endosperm, phytate is probably hydrolyzed by phytase and the released Fe bound to NA. As  
480 in dicotyledonous species, the precise phytase involved have not yet been identified. Then, the  
481 main question is how is Fe taken up from the endosperm into the embryo and further  
482 distributed to growing organs within the embryo. Time course analysis of gene expression and  
483 Fe localization during rice germination has highlighted several candidates<sup>94, 120</sup>. X-ray  
484 fluorescence imaging revealed that after 24h of germination, Fe accumulates in the epithelium  
485 and scutellum, the Fe level decreases in the scutellum and increases in the coleoptile. Iron is  
486 already visible in the root tip after 36 hours<sup>94</sup>. Genes encoding NA and phytosiderophore  
487 biosynthesis were upregulated during germination<sup>120</sup>. Interestingly, NA synthase expression  
488 was prominent in the endosperm while NA amino transferase (NAAT) was expressed in the  
489 embryo. Accordingly, several members of the YSL NA and phytosiderophore transporters,  
490 YSL2, 6, 10, 12 and 14 are upregulated<sup>120</sup>. The expression of TOM2, a NA and  
491 phytosiderophore efflux transporter and of OsYSL2 increased markedly in the scutellum  
492 during germination<sup>19, 120</sup>. These data indicate that Fe is transported as a complex with NA  
493 into the embryo or phytosiderophores within the embryo. Several transporters for free divalent  
494 Fe were also upregulated during germination: OsIRT1, several OsNramp genes and a  
495 Ferroportin/IREG<sup>94, 120</sup>. As in dicotyledonous species, the citrate efflux transporter OsFRDL1  
496 is upregulated indicating that citrate is also secreted to maintain Fe solubility during cell-to-  
497 cell transport<sup>34, 35</sup>. Finally, the rice homologue of the chloroplast Fe influx system in  
498 Arabidopsis PIC1 is upregulated after 3 days probably to sustain the differentiation of plastids  
499 into chloroplast during early seedling development<sup>121</sup>. Many transporters of Fe<sup>2+</sup> or Fe  
500 complexes with NA or siderophores are apparently involved in Fe remobilization after grain  
501 germination. However, the individual contribution of each of these transporters remains to be  
502 analyzed to identify the key steps in Fe remobilization in graminaceous species.

503 With its uptake into chloroplasts of the developing seedling, Fe completes its journey from the  
504 senescing leaves of the mother plant to the expanding photosynthetic apparatus of its progeny,  
505 via the seed.

506

507 **Conclusions and perspectives**



508 Many transporters and ligands for Fe have been characterized and this knowledge has been  
509 used to design targeted biofortification strategies. However, many key steps remain to be  
510 elucidated at the molecular level. The mechanisms that make iron available for reallocation to  
511 the seeds during leaf senescence need to be explored in more details. It would be important to  
512 determine which of the different pathways that contribute to chloroplast degradation are most  
513 important for Fe mobilization. Important questions also still need to be answered concerning  
514 phloem transport: the ligand for Fe in the phloem is still not unequivocally identified.  
515 Moreover, whereas OPT3 plays a key role in Fe transport to seeds, the specific substrate of  
516 this transporter is still unknown. The transfer of Fe from the mother plant to the embryo has  
517 been under investigated so far and probably deserves more attention (Figure 2). The  
518 pioneering discovery that ascorbic acid is responsible for Fe reduction in the extracellular  
519 space separating the mother tissues from the embryo raises important questions. How general  
520 this mechanism is remains to be determined. For example, the relevance of this finding for  
521 grain staples needs to be addressed. There is also a need to identify the molecular players  
522 involved in ascorbate secretion and regeneration. Moreover, even though FRD3 has been  
523 proposed to be responsible for citrate efflux in the intracellular space separating the maternal  
524 tissues and the embryo, this hypothesis would need to be substantiated and other transporters  
525 potentially involved in citrate and malate efflux need to be identified. Last but not least, the  
526 transport mechanisms responsible for Fe efflux from mother tissues and reuptake into the  
527 embryo remain completely unknown. Tissue specific transcriptomic analyses have provided  
528 candidates for mediating these steps in barley<sup>68</sup>. The role of each candidate has now to be  
529 analyzed in detail and similar analyses are lacking in Arabidopsis or other dicots.  
530 Manipulating these steps would certainly open new perspectives for biofortification. Besides  
531 the effectors of Fe transport and complexation during Fe loading into seeds, it would also be  
532 important to get insights into the master regulators that control Fe storage in seeds. How Fe  
533 storage is connected to the signaling pathways and transcription factors that regulate seed  
534 development, such as Leafy Cotyledon 2 (LEC2) or WRINCKLED, needs to be unraveled<sup>122</sup>.  
535 When considering Fe remobilization after germination, many questions are still open. In  
536 Arabidopsis and in dicots in general, several steps upstream and downstream of the action of  
537 vacuolar efflux by NRAMP3/4 remain to be investigated. The molecular identity of the  
538 phytases that make Fe available for transport has to be determined. Whether Fe needs to be  
539 reduced prior to transport by vacuolar NRAMP and by which mechanism are still open  
540 questions. Downstream of vacuolar efflux the plasma membrane transporters that allow Fe  
541 efflux from endodermal cells to other cell types, as well as the mechanisms that allow Fe

542 distribution to plastids, are still to be identified. Similar questions arise concerning the use of  
543 Fe stored in aleurone cells after the germination of grains of monocots. Finally, the master  
544 regulators that orchestrate Fe remobilization in response to hormonal cues, such as  
545 gibberellins, also need to be defined.

546 In conclusion, even though many players involved in Fe storage and remobilization from  
547 seeds have been discovered in the last decades, we still lack a molecular understanding of  
548 several key steps. In addition to improving our basic knowledge of how plants transfer  
549 nutrients from one generation to the next, unraveling these steps holds great promises for seed  
550 Fe biofortification and possibly for improving seed quality and stand establishment.

551

## 552 **Acknowledgements**

553 The work on seed iron storage in ST, SM and CB laboratories is supported by the CNRS, the  
554 INRA and Paris Sorbonne University, as well as by the collaborative ANR grant ISISTOR  
555 (ANR-16-CE20-0019-02).

556

## 557 **References**

558

- 559 1. Have, M., et al., (2017) Nitrogen remobilization during leaf senescence: lessons from  
560 Arabidopsis to crops. *J Exp Bot.* **68**(10): p. 2513-2529.
- 561 2. Pottier, M., et al., (2019) Autophagy is essential for optimal translocation of iron to seeds in  
562 Arabidopsis. *J Exp Bot.* **70**(3): p. 859-869.
- 563 3. Waters, B.M. and M.A. Grusak, (2008) Whole-plant mineral partitioning throughout the life  
564 cycle in Arabidopsis thaliana ecotypes Columbia, Landsberg erecta, Cape Verde Islands, and  
565 the mutant line ysl1ysl3. *New Phytol.* **177**(2): p. 389-405.
- 566 4. Murgia, I., et al., (2012) Biofortification for combating 'hidden hunger' for iron. *Trends Plant*  
567 *Sci.* **17**(1): p. 47-55.
- 568 5. Grillet, L., S. Mari, and W. Schmidt, (2014) Iron in seeds - loading pathways and subcellular  
569 localization. *Front Plant Sci.* **4**: p. 535.
- 570 6. Connorton, J.M., J. Balk, and J. Rodriguez-Celma, (2017) Iron homeostasis in plants - a brief  
571 overview. *Metallomics.* **9**(7): p. 813-823.
- 572 7. Kobayashi, T. and N.K. Nishizawa, (2012) Iron uptake, translocation, and regulation in higher  
573 plants. *Annu Rev Plant Biol.* **63**: p. 131-52.
- 574 8. Santi, S. and W. Schmidt, (2009) Dissecting iron deficiency-induced proton extrusion in  
575 Arabidopsis roots. *New Phytol.* **183**(4): p. 1072-84.
- 576 9. Rajniak, J., et al., (2018) Biosynthesis of redox-active metabolites in response to iron  
577 deficiency in plants. *Nat Chem Biol.* **14**(5): p. 442-450.
- 578 10. Tsai, H.H. and W. Schmidt, (2017) Mobilization of Iron by Plant-Borne Coumarins. *Trends*  
579 *Plant Sci.* **22**(6): p. 538-548.
- 580 11. Robinson, N.J., et al., (1999) A ferric-chelate reductase for iron uptake from soils. *Nature*  
581 (London). **397**(6721): p. 694-697.
- 582 12. Vert, G., et al., (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil  
583 and for plant growth. *Plant Cell.* **14**(6): p. 1223-33.
- 584 13. Connolly, E.L., J.P. Fett, and M.-L. Guerinot, (2002) Expression of the IRT1 metal transporter is  
585 controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell.* **14**:  
586 p. 1347-1357.

- 587 14. Eide, D., et al., (1996) A novel iron-regulated metal transporter from plants identified by  
588 functional expression in yeast. Proceedings of the National Academy of Sciences of the  
589 United States of America. **93**(11): p. 5624-5628.
- 590 15. Korshunova, Y.O., et al., (1999) The *IRT1* protein from *Arabidopsis thaliana* is a metal  
591 transporter with a broad substrate range. Plant Molecular Biology. **40**(1): p. 37-44.
- 592 16. Dubeaux, G., et al., (2018) Metal Sensing by the IRT1 Transporter-Receptor Orchestrates Its  
593 Own Degradation and Plant Metal Nutrition. Mol Cell. **69**(6): p. 953-964 e5.
- 594 17. Thomine, S. and G. Vert, (2013) Iron transport in plants: better be safe than sorry. Curr Opin  
595 Plant Biol. **16**(3): p. 322-7.
- 596 18. Chan-Rodriguez, D. and E.L. Walker, (2018) Analysis of Yellow Striped Mutants of *Zea mays*  
597 Reveals Novel Loci Contributing to Iron Deficiency Chlorosis. Front Plant Sci. **9**: p. 157.
- 598 19. Nozoye, T., et al., (2015) The Phytosiderophore Efflux Transporter TOM2 Is Involved in Metal  
599 Transport in Rice. J Biol Chem. **290**(46): p. 27688-99.
- 600 20. Curie, C., et al., (2001) Maize yellow stripe1 encodes a membrane protein directly involved in  
601 Fe(III) uptake. Nature. **409**(6818): p. 346-349.
- 602 21. Inoue, H., et al., (2009) Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid  
603 transporter expressed in the roots and is essential for iron uptake in early growth of the  
604 seedlings. J Biol Chem. **284**(6): p. 3470-9.
- 605 22. Lee, S., et al., (2009) Disruption of OsYSL15 leads to iron inefficiency in rice plants. Plant  
606 Physiol. **150**(2): p. 786-800.
- 607 23. Gao, F., et al., (2019) The Transcriptional Control of Iron Homeostasis in Plants: A Tale of  
608 bHLH Transcription Factors? Front Plant Sci. **10**: p. 6.
- 609 24. Kobayashi, T., T. Nozoye, and N.K. Nishizawa, (2019) Iron transport and its regulation in  
610 plants. Free Radic Biol Med. **133**: p. 11-20.
- 611 25. Kobayashi, T., et al., (2013) Iron-binding haemerythrin RING ubiquitin ligases regulate plant  
612 iron responses and accumulation. Nat Commun. **4**: p. 2792.
- 613 26. Long, T.A., et al., (2010) The bHLH transcription factor POPEYE regulates response to iron  
614 deficiency in *Arabidopsis* roots. Plant Cell. **22**(7): p. 2219-36.
- 615 27. Rodriguez-Celma, J., et al., (2019) *Arabidopsis* BRUTUS-LIKE E3 ligases negatively regulate iron  
616 uptake by targeting transcription factor FIT for recycling. Proc Natl Acad Sci U S A.
- 617 28. Barberon, M., (2017) The endodermis as a checkpoint for nutrients. New Phytol. **213**(4): p.  
618 1604-1610.
- 619 29. Barberon, M., et al., (2016) Adaptation of Root Function by Nutrient-Induced Plasticity of  
620 Endodermal Differentiation. Cell. **164**(3): p. 447-59.
- 621 30. Morrissey, J., et al., (2009) The ferroportin metal efflux proteins function in iron and cobalt  
622 homeostasis in *Arabidopsis*. Plant Cell. **21**(10): p. 3326-38.
- 623 31. Durrett, T.P., W. Gassmann, and E.E. Rogers, (2007) The FRD3-mediated efflux of citrate into  
624 the root vasculature is necessary for efficient iron translocation. Plant Physiol. **144**(1): p. 197-  
625 205.
- 626 32. Flis, P., et al., (2016) Inventory of metal complexes circulating in plant fluids: a reliable  
627 method based on HPLC coupled with dual elemental and high-resolution molecular mass  
628 spectrometric detection. New Phytol. **211**(3): p. 1129-41.
- 629 33. Rogers, E.E. and M.L. Guerinot, (2002) FRD3, a member of the multidrug and toxin efflux  
630 family, controls iron deficiency responses in *Arabidopsis*. Plant Cell. **14**(8): p. 1787-99.
- 631 34. Roschttardt, H., et al., (2011) The FRD3 citrate effluxer promotes iron nutrition between  
632 symplastically disconnected tissues throughout *Arabidopsis* development. Plant Cell. **23**(7): p.  
633 2725-37.
- 634 35. Yokosho, K., et al., (2009) OsFRDL1 is a citrate transporter required for efficient translocation  
635 of iron in rice. Plant Physiol. **149**(1): p. 297-305.
- 636 36. Lanquar, V., et al., (2010) Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is  
637 required for optimal photosynthesis and growth under manganese deficiency. Plant Physiol.  
638 **152**(4): p. 1986-99.

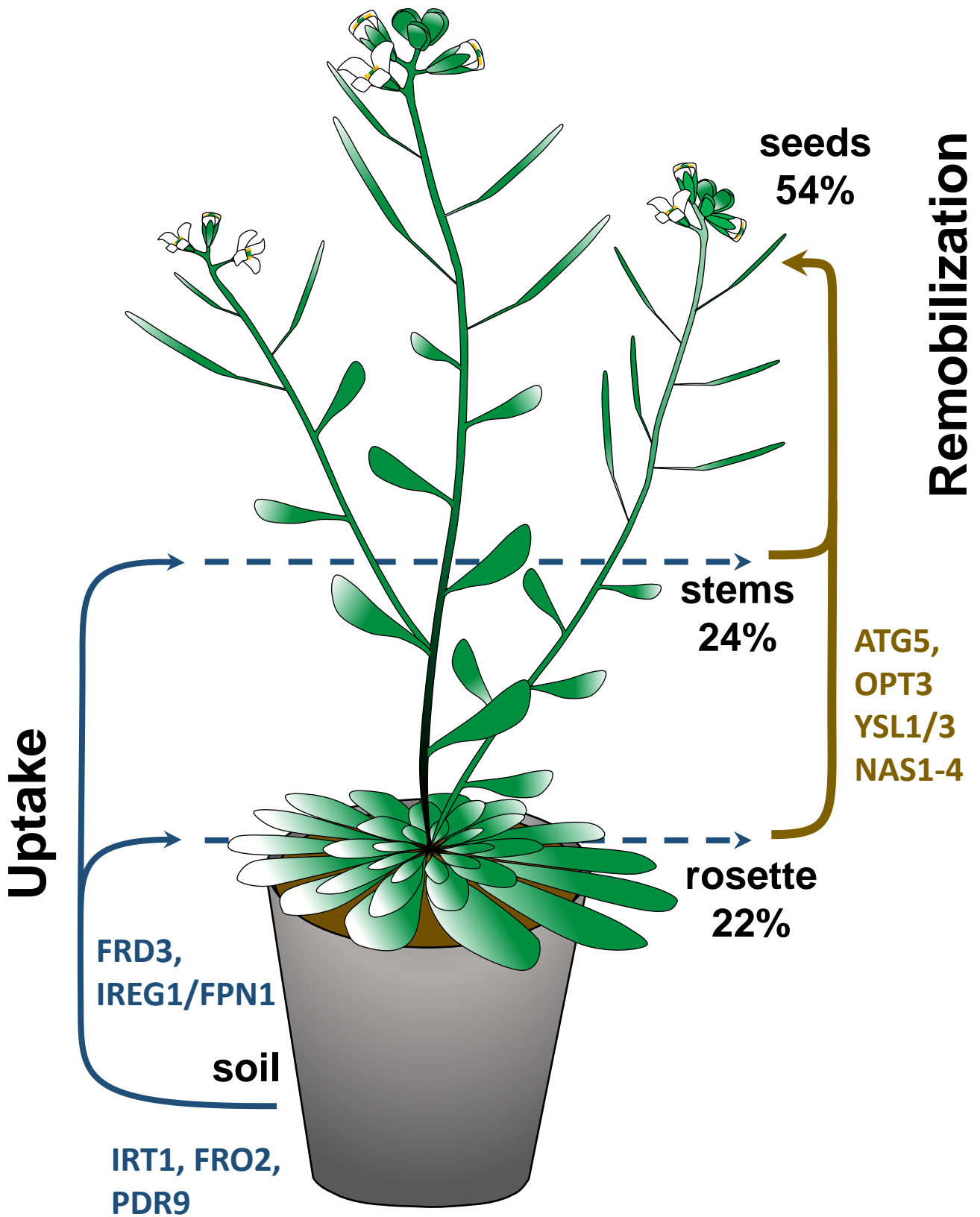
- 639 37. Roschztardt, H., et al., (2013)New insights into Fe localization in plant tissues. *Front Plant*  
640 *Sci.* **4**: p. 350.
- 641 38. Shingles, R., M. North, and R.E. McCarty, (2002)Ferrous ion transport across chloroplast inner  
642 envelope membranes. *Plant Physiol.* **128**(3): p. 1022-30.
- 643 39. Pottier, M., et al., (2014)Autophagy as a possible mechanism for micronutrient  
644 remobilization from leaves to seeds. *Front Plant Sci.* **5**: p. 11.
- 645 40. Guiboileau, A., et al., (2012)Autophagy machinery controls nitrogen remobilization at the  
646 whole-plant level under both limiting and ample nitrate conditions in Arabidopsis. *New*  
647 *Phytol.* **194**(3): p. 732-40.
- 648 41. Otegui, M.S., (2018)Vacuolar degradation of chloroplast components: autophagy and  
649 beyond. *J Exp Bot.* **69**(4): p. 741-750.
- 650 42. Izumi, M., et al., (2017)Entire Photodamaged Chloroplasts Are Transported to the Central  
651 Vacuole by Autophagy. *Plant Cell.* **29**(2): p. 377-394.
- 652 43. Michaeli, S., et al., (2014)Arabidopsis ATG8-INTERACTING PROTEIN1 is involved in autophagy-  
653 dependent vesicular trafficking of plastid proteins to the vacuole. *Plant Cell.* **26**(10): p. 4084-  
654 101.
- 655 44. Wang, S. and E. Blumwald, (2014)Stress-induced chloroplast degradation in Arabidopsis is  
656 regulated via a process independent of autophagy and senescence-associated vacuoles. *Plant*  
657 *Cell.* **26**(12): p. 4875-88.
- 658 45. Qiu, K., et al., (2015)EIN3 and ORE1 Accelerate Degreening during Ethylene-Mediated Leaf  
659 Senescence by Directly Activating Chlorophyll Catabolic Genes in Arabidopsis. *PLoS Genet.*  
660 **11**(7): p. e1005399.
- 661 46. Ricachenevsky, F.K., P.K. Menguer, and R.A. Sperotto, (2013)kNACKing on heaven's door:  
662 how important are NAC transcription factors for leaf senescence and Fe/Zn remobilization to  
663 seeds? *Front Plant Sci.* **4**: p. 226.
- 664 47. Uauy, C., et al., (2006)A NAC Gene regulating senescence improves grain protein, zinc, and  
665 iron content in wheat. *Science.* **314**(5803): p. 1298-301.
- 666 48. Klatte, M., et al., (2009)The analysis of Arabidopsis nicotianamine synthase mutants reveals  
667 functions for nicotianamine in seed iron loading and iron deficiency responses. *Plant Physiol.*  
668 **150**(1): p. 257-71.
- 669 49. Ling, H.Q., et al., (1999)Map-based cloning of chloronerva, a gene involved in iron uptake of  
670 higher plants encoding nicotianamine synthase. *Proceedings of the National Academy of*  
671 *Sciences of the United States of America.* **96**: p. 7098-103.
- 672 50. Takahashi, M., et al., (2003)Role of nicotianamine in the intracellular delivery of metals and  
673 plant reproductive development. *Plant Cell.* **15**(6): p. 1263-80.
- 674 51. Koike, S., et al., (2004)OsYSL2 is a rice metal-nicotianamine transporter that is regulated by  
675 iron and expressed in the phloem. *Plant J.* **39**(3): p. 415-24.
- 676 52. Ishimaru, Y., et al., (2010)Rice metal-nicotianamine transporter, OsYSL2, is required for the  
677 long-distance transport of iron and manganese. *Plant J.* **62**(3): p. 379-90.
- 678 53. Senoura, T., et al., (2017)The iron-chelate transporter OsYSL9 plays a role in iron distribution  
679 in developing rice grains. *Plant Mol Biol.* **95**(4-5): p. 375-387.
- 680 54. Chu, H.H., et al., (2010)Successful reproduction requires the function of Arabidopsis Yellow  
681 Stripe-Like1 and Yellow Stripe-Like3 metal-nicotianamine transporters in both vegetative and  
682 reproductive structures. *Plant Physiol.* **154**(1): p. 197-210.
- 683 55. Le Jean, M., et al., (2005)A loss-of-function mutation in AtYSL1 reveals its role in iron and  
684 nicotianamine seed loading. *Plant J.* **44**(5): p. 769-82.
- 685 56. Waters, B.M., et al., (2006)Mutations in Arabidopsis yellow stripe-like1 and yellow stripe-  
686 like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant*  
687 *Physiol.* **141**(4): p. 1446-58.
- 688 57. Schuler, M., et al., (2012)Nicotianamine functions in the Phloem-based transport of iron to  
689 sink organs, in pollen development and pollen tube growth in Arabidopsis. *Plant Cell.* **24**(6):  
690 p. 2380-400.

- 691 58. Stacey, M.G., et al., (2002)AtOPT3, a member of the oligopeptide transporter family, is  
692 essential for embryo development in Arabidopsis. *Plant Cell*. **14**(11): p. 2799-811.
- 693 59. Bogs, J., et al., (2003)Functional characterization and expression analysis of a glutathione  
694 transporter, BjGT1, from Brassica juncea: evidence for regulation by heavy metal exposure.  
695 *Plant Cell Environ*. **26**: p. 1703-1711.
- 696 60. Zhai, Z., et al., (2014)OPT3 Is a Phloem-Specific Iron Transporter That Is Essential for Systemic  
697 Iron Signaling and Redistribution of Iron and Cadmium in Arabidopsis. *Plant Cell*. **26**(5): p.  
698 2249-2264.
- 699 61. Stacey, M.G., et al., (2008)The Arabidopsis AtOPT3 protein functions in metal homeostasis  
700 and movement of iron to developing seeds. *Plant Physiol*. **146**(2): p. 589-601.
- 701 62. Mendoza-Cozatl, D.G., et al., (2014)OPT3 is a component of the iron-signaling network  
702 between leaves and roots and misregulation of OPT3 leads to an over-accumulation of  
703 cadmium in seeds. *Mol Plant*. **7**(9): p. 1455-1469.
- 704 63. Bashir, K., et al., (2015)Iron deficiency regulated OsOPT7 is essential for iron homeostasis in  
705 rice. *Plant Mol Biol*. **88**(1-2): p. 165-76.
- 706 64. Grillet, L., et al., (2014)Ascorbate efflux as a new strategy for iron reduction and transport in  
707 plants. *J Biol Chem*. **289**(5): p. 2515-25.
- 708 65. Jain, A., G.T. Wilson, and E.L. Connolly, (2014)The diverse roles of FRO family  
709 metalloreductases in iron and copper homeostasis. *Front Plant Sci*. **5**: p. 100.
- 710 66. Jeong, J., et al., (2008)Chloroplast Fe(III) chelate reductase activity is essential for seedling  
711 viability under iron limiting conditions. *Proc Natl Acad Sci U S A*. **105**(30): p. 10619-24.
- 712 67. Olsen, L.I., et al., (2016)Mother-plant-mediated pumping of zinc into the developing seed.  
713 *Nat Plants*. **2**(5): p. 16036.
- 714 68. Borg, S., et al., (2009)Iron Transport, deposition and bioavailability in the wheat and barley  
715 grain. *Plant Soil*. **325**: p. 15-24.
- 716 69. De Brier, N., et al., (2016)Element distribution and iron speciation in mature wheat grains  
717 (*Triticum aestivum* L.) using synchrotron X-ray fluorescence microscopy mapping and X-ray  
718 absorption near-edge structure (XANES) imaging. *Plant Cell Environ*. **39**(8): p. 1835-47.
- 719 70. Connorton, J.M., et al., (2017)Wheat Vacuolar Iron Transporter TaVIT2 Transports Fe and Mn  
720 and Is Effective for Biofortification. *Plant Physiol*. **174**(4): p. 2434-2444.
- 721 71. Kim, S.A., et al., (2006)Localization of iron in Arabidopsis seed requires the vacuolar  
722 membrane transporter VIT1. *Science*. **314**(5803): p. 1295-8.
- 723 72. Lott, J.N. and E. Spitzer, (1980)X-ray Analysis Studies of Elements Stored in Protein Body  
724 Globoid Crystals of *Triticum* Grains. *Plant Physiol*. **66**(3): p. 494-9.
- 725 73. Curie, C., et al., (2009)Metal movement within the plant: contribution of nicotianamine and  
726 yellow stripe 1-like transporters. *Ann Bot*. **103**(1): p. 1-11.
- 727 74. Clemens, S., (2014)Zn and Fe biofortification: the right chemical environment for human  
728 bioavailability. *Plant Sci*. **225**: p. 52-7.
- 729 75. Hallberg, L., (2001)Perspective on nutritional iron deficiency. *Annual Review of Nutrition*. **21**:  
730 p. 1-21.
- 731 76. Briat, J.F., (1999)Plant ferritin and human iron deficiency. *Nat Biotechnol*. **17**(7): p. 621.
- 732 77. Chu, H.H., et al., (2017)The Arabidopsis MTP8 transporter determines the localization of  
733 manganese and iron in seeds. *Sci Rep*. **7**(1): p. 11024.
- 734 78. Eroglu, S., et al., (2017)Metal Tolerance Protein 8 Mediates Manganese Homeostasis and  
735 Iron Reallocation during Seed Development and Germination. *Plant Physiol*. **174**(3): p. 1633-  
736 1647.
- 737 79. Mary, V., et al., (2015)Bypassing Iron Storage in Endodermal Vacuoles Rescues the Iron  
738 Mobilization Defect in the natural resistance associated-macrophage protein3natural  
739 resistance associated-macrophage protein4 Double Mutant. *Plant Physiol*. **169**(1): p. 748-59.
- 740 80. Roschttardt, H., et al., (2009)Identification of the endodermal vacuole as the iron storage  
741 compartment in the Arabidopsis embryo. *Plant Physiol*. **151**(3): p. 1329-38.

- 742 81. Schnell Ramos, M., et al., (2013)Using muPIXE for quantitative mapping of metal  
743 concentration in Arabidopsis thaliana seeds. *Front Plant Sci.* **4**: p. 168.
- 744 82. Lanquar, V., et al., (2005)Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is  
745 essential for seed germination on low iron. *EMBO J.* **24**(23): p. 4041-51.
- 746 83. Ibeas, M.A., et al., (2017)Dynamic Subcellular Localization of Iron during Embryo  
747 Development in Brassicaceae Seeds. *Front Plant Sci.* **8**: p. 2186.
- 748 84. Roschztardt, H., et al., (2011)Plant cell nucleolus as a hot spot for iron. *J Biol Chem.*  
749 **286**(32): p. 27863-6.
- 750 85. Moore, K.L., et al., (2018)The stage of seed development influences iron bioavailability in pea  
751 (*Pisum sativum* L.). *Sci Rep.* **8**(1): p. 6865.
- 752 86. Cvitanich, C., et al., (2010)Iron and ferritin accumulate in separate cellular locations in  
753 *Phaseolus* seeds. *BMC Plant Biol.* **10**: p. 26.
- 754 87. Eroglu, S., et al., (2019)The Conservation of VIT1-Dependent Iron Distribution in Seeds. *Front*  
755 *Plant Sci.* **10**: p. 907.
- 756 88. Ibeas, M.A., et al., (2018)The Diverse Iron Distribution in Eudicotyledoneae Seeds: From  
757 *Arabidopsis* to Quinoa. *Front Plant Sci.* **9**: p. 1985.
- 758 89. Fath, A., P.C. Bethke, and R.L. Jones, (1999)Barley aleurone cell death is not apoptotic:  
759 characterization of nuclease activities and DNA degradation. *Plant J.* **20**(3): p. 305-15.
- 760 90. Jones, R.L., (1969)Gibberellic acid and the fine structure of barley aleurone cells : II. Changes  
761 during the synthesis and secretion of alpha-amylase. *Planta.* **88**(1): p. 73-86.
- 762 91. Iwai, T., et al., (2012)Dynamic changes in the distribution of minerals in relation to phytic acid  
763 accumulation during rice seed development. *Plant Physiol.* **160**(4): p. 2007-14.
- 764 92. Singh, S.P., et al., (2013)Pattern of iron distribution in maternal and filial tissues in wheat  
765 grains with contrasting levels of iron. *J Exp Bot.* **64**(11): p. 3249-60.
- 766 93. De Brier, N., et al., (2015)Distribution of Minerals in Wheat Grains (*Triticum aestivum* L.) and  
767 in Roller Milling Fractions Affected by Pearling. *J Agric Food Chem.* **63**(4): p. 1276-1285.
- 768 94. Takahashi, M., et al., (2009)In vivo analysis of metal distribution and expression of metal  
769 transporters in rice seed during germination process by microarray and X-ray Fluorescence  
770 Imaging of Fe, Zn, Mn, and Cu. *Plant and Soil.* **325**: p. 39-51.
- 771 95. Lee, S., et al., (2009)Iron fortification of rice seeds through activation of the nicotianamine  
772 synthase gene. *Proc Natl Acad Sci U S A.* **106**(51): p. 22014-9.
- 773 96. Eagling, T., et al., (2014)Distribution and speciation of iron and zinc in grain of two wheat  
774 genotypes. *J Agric Food Chem.* **62**(3): p. 708-16.
- 775 97. Bashir, K., et al., (2013)The knockdown of OsVIT2 and MIT affects iron localization in rice  
776 seed. *Rice (N Y).* **6**(1): p. 31.
- 777 98. Zhang, Y., et al., (2012)Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron  
778 translocation between flag leaves and seeds in rice. *Plant J.* **72**(3): p. 400-10.
- 779 99. Kawakami, Y. and N.K. Bhullar, (2018)Molecular processes in iron and zinc homeostasis and  
780 their modulation for biofortification in rice. *J Integr Plant Biol.* **60**(12): p. 1181-1198.
- 781 100. Bashir, K., et al., (2013)Exploiting new tools for iron bio-fortification of rice. *Biotechnol Adv.*  
782 **31**(8): p. 1624-33.
- 783 101. Connorton, J.M. and J. Balk, (2019)Iron Biofortification of Staple Crops: Lessons and  
784 Challenges in Plant Genetics. *Plant Cell Physiol.* **60**(7): p. 1447-1456.
- 785 102. Goto, F., et al., (1999)Iron fortification of rice seed by the soybean ferritin gene. *Nat*  
786 *Biotechnol.* **17**(3): p. 282-6.
- 787 103. Qu le, Q., et al., (2005)Iron accumulation does not parallel the high expression level of ferritin  
788 in transgenic rice seeds. *Planta.* **222**(2): p. 225-33.
- 789 104. Wu, T.Y., W. Gruissem, and N.K. Bhullar, (2019)Targeting intracellular transport combined  
790 with efficient uptake and storage significantly increases grain iron and zinc levels in rice.  
791 *Plant Biotechnol J.* **17**(1): p. 9-20.
- 792 105. Bewley, J.D., (1997)Seed Germination and Dormancy. *Plant Cell.* **9**(7): p. 1055-1066.

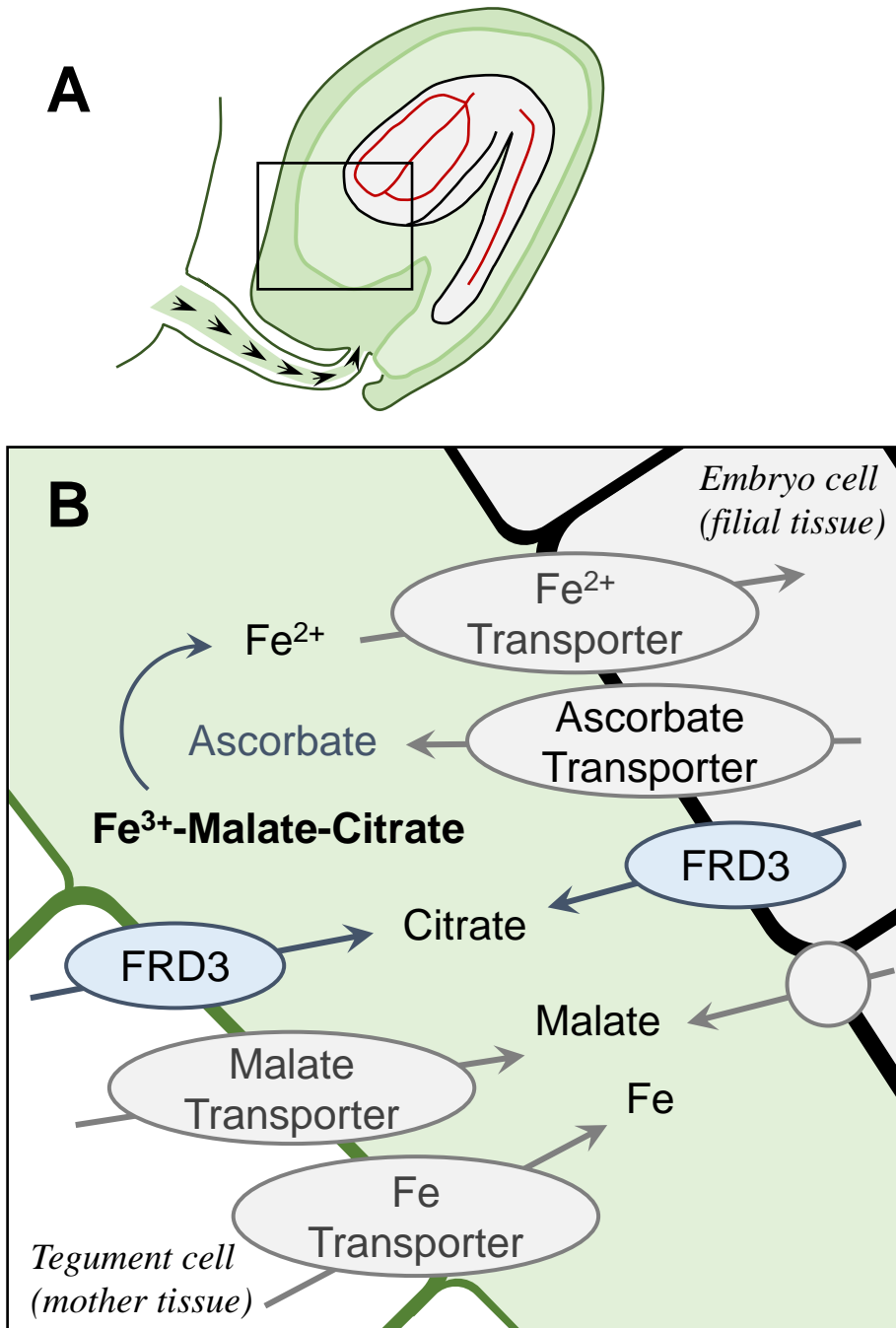
- 793 106. Finch-Savage, W.E. and G. Leubner-Metzger, (2006)Seed dormancy and the control of  
794 germination. *New Phytol.* **171**(3): p. 501-23.
- 795 107. Li, X., et al., (2012)Responses of seedling growth and antioxidant activity to excess iron and  
796 copper in *Triticum aestivum* L. *Ecotoxicol Environ Saf.* **86**: p. 47-53.
- 797 108. Nozoe, T., et al., (2009)Effects of ferrous iron (Fe) on the germination and root elongation of  
798 paddy rice and weeds. *Weed Biol. Manag.* **9**: p. 20–26.
- 799 109. Afify Ael, M., et al., (2011)Bioavailability of iron, zinc, phytate and phytase activity during  
800 soaking and germination of white sorghum varieties. *PLoS One.* **6**(10): p. e25512.
- 801 110. Azeke, M.A., et al., (2011)Effect of germination on the phytase activity, phytate and total  
802 phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*),  
803 sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). *J Food Sci Technol.* **48**(6): p. 724-  
804 9.
- 805 111. Tian, B., et al., (2010)Physicochemical changes of oat seeds during germination. *Food*  
806 *Chemistry.* **119**: p. 1195–1200.
- 807 112. Müller, K., et al., ((2009)In Vivo Cell Wall Loosening by Hydroxyl Radicals during Cress Seed  
808 Germination and Elongation Growth. *Plant Physiol.* **150**: p. 1855–1865.
- 809 113. Murgia, I. and P. Morandini, (2017)Iron Deficiency Prolongs Seed Dormancy in *Arabidopsis*  
810 *Plants.* *Front Plant Sci.* **8**: p. 2077.
- 811 114. Bailly, C., (2004) Active oxygen species and antioxidants in seed biology. *Seed Science*  
812 *Research.* **14**: p. 93–107.
- 813 115. Bailly, C., H. El-Maarouf-Bouteau, and F. Corbineau, (2008)From intracellular signaling  
814 networks to cell death: the dual role of reactive oxygen species in seed physiology. *C R Biol.*  
815 **331**(10): p. 806-14.
- 816 116. El-Maarouf-Bouteau, H., et al., (2015)Reactive oxygen species, abscisic acid and ethylene  
817 interact to regulate sunflower seed germination. *Plant Cell Environ.* **38**(2): p. 364-74.
- 818 117. Leymarie, J., et al., (2012)Role of reactive oxygen species in the regulation of *Arabidopsis*  
819 seed dormancy. *Plant Cell Physiol.* **53**(1): p. 96-106.
- 820 118. Bastow, E.L., et al., (2018)Vacuolar Iron Stores Gated by NRAMP3 and NRAMP4 Are the  
821 Primary Source of Iron in Germinating Seeds. *Plant Physiol.* **177**(3): p. 1267-1276.
- 822 119. Belgaroui, N., et al., (2014)Over-expression of the bacterial phytase US417 in *Arabidopsis*  
823 reduces the concentration of phytic acid and reveals its involvement in the regulation of  
824 sulfate and phosphate homeostasis and signaling. *Plant Cell Physiol.* **55**(11): p. 1912-24.
- 825 120. Nozoye, T., et al., (2007)The expression of iron homeostasis-related genes during rice  
826 germination. *Plant Mol Biol.* **64**(1-2): p. 35-47.
- 827 121. Duy, D., et al., (2007)PIC1, an ancient permease in *Arabidopsis* chloroplasts, mediates iron  
828 transport. *Plant Cell.* **19**(3): p. 986-1006.
- 829 122. Baud, S., et al., (2016)Deciphering the Molecular Mechanisms Underpinning the  
830 Transcriptional Control of Gene Expression by Master Transcriptional Regulators in  
831 *Arabidopsis* Seed. *Plant Physiol.* **171**(2): p. 1099-112.

832

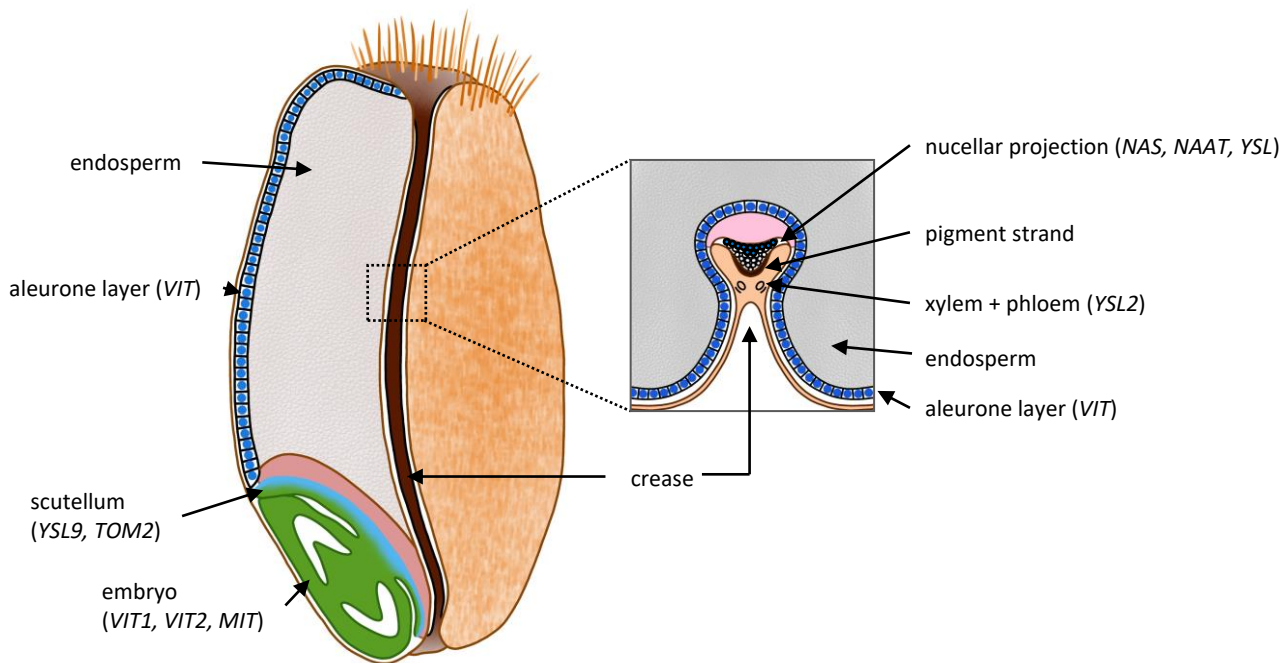


**Figure 1:** Fe uptake and remobilization to seeds in *Arabidopsis thaliana*. The names of the main proteins involved in uptake and translocation from roots to shoots are indicated in blue. The name of the main proteins involved in remobilization to seeds are indicated in brown. The percentages indicate the mean distribution of Fe at the end of the plant's life cycle, excluding the roots (adapted from Pottier et al., 2019)

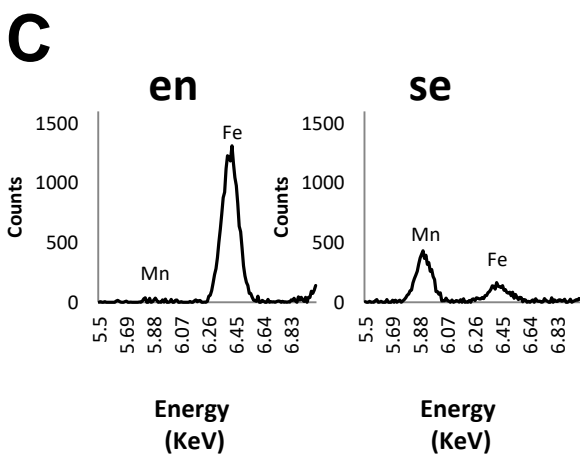
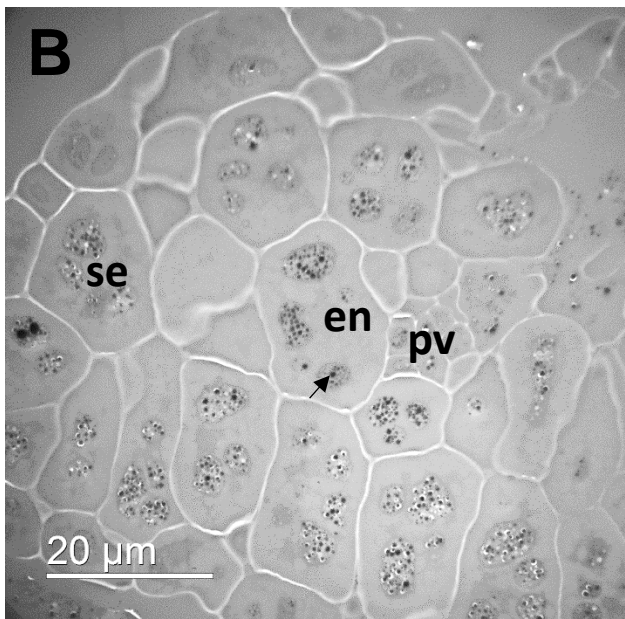




**Figure 2:** Fe transfer from the maternal to embryo tissues. A. Fe is transported to the maternal tissues of the developing seed but has to cross an extracellular space between the teguments and the embryo. B. Fe is bound to malate and citrate in the extracellular space separating the maternal tissues from the embryo. In Arabidopsis and pea, Fe is reduced by ascorbate prior to its uptake into the embryo. The transporters responsible for Fe, malate and ascorbate secretion to the extracellular space as well as the transporter responsible for Fe uptake into the embryo remain to be identified.



**Figure 3:** Fe distribution in a mature cereal grain (eg wheat grain). Fe-rich tissues and cells appear in blue, symplastic disconnections (between maternal tissues and endosperm; between endosperm and scutellum) are highlighted in pink. The size, shape and development of the crease and the nucellar projection vary among the monocot phylogeny.



**Figure 4:** Fe distribution in *Arabidopsis thaliana* mature embryo. A. Perls DAB staining of Fe in the whole embryo shows high concentration of Fe (brown color) around the pro-vasculature. B. TEM micrograph of an embryo section in the cotyledon showing the pro-vasculature (pv), proto-endodermis cells (en) and subepidermal cells (se). The phytate globoids appear as small black dots inside the storage vacuole (arrow). C. EDX analysis of protein bodies in proto-endodermal (en) or sub-epidermal cells (se): Fe is concentrated in the storage vacuole of proto-endodermal cells (en).