



Increasing Provasculature Complexity in the Arabidopsis Embryo May Increase Total Iron Content in Seeds: A Hypothesis

Hannetz Roschztardt^{*}, Sofia Bustos, Maria F. Coronas, Miguel A. Ibeas, Susana Grant-Grant and Joaquín Vargas-Pérez

Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

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*Correspondence:

Hannetz Roschztardt
hroschztardt@bio.puc.cl

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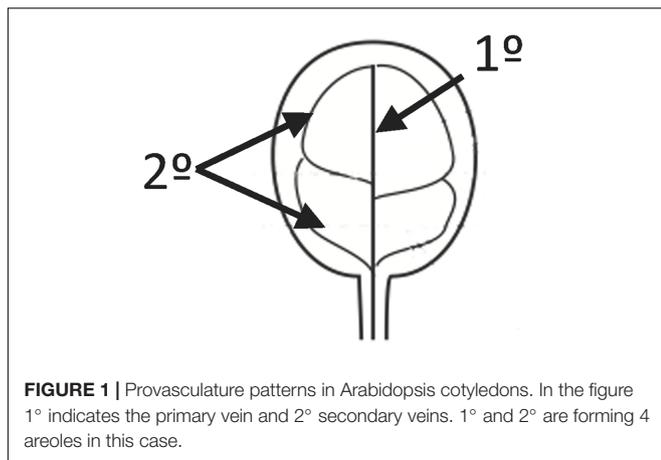
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Anemia due to iron deficiency is a worldwide issue, affecting mainly children and women. Seed iron is a major source of this micronutrient for feeding, however, in most crops these levels are too low to meet daily needs. Thus, increasing iron allocation and its storage in seeds can represent an important step to enhance iron provision for humans and animals. Our knowledge on seed iron homeostasis is mainly based on studies performed in the model plant *Arabidopsis thaliana*, where iron accumulates in endodermis cells surrounding the embryo provasculature. It has been reported that cotyledon provasculature pattern complexity can be modified, thus we hypothesize that changes in the complexity of embryo vein patterns may affect total iron content in Arabidopsis seeds. This approach could be used as basis to develop strategies aimed to biofortify seeds.

Keywords: iron, embryo, endodermis, provasculature, biofortification

INTRODUCTION

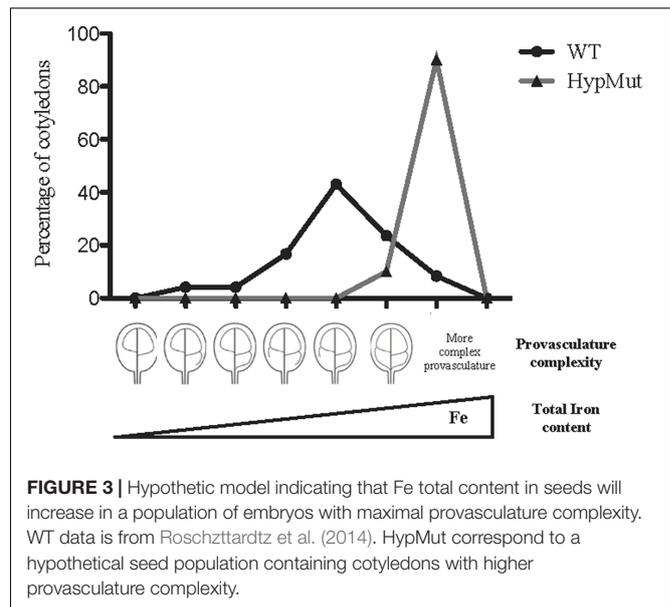
Malnutrition is one of the greatest health problems in the world, causing chronic diseases, altered physical and mental development, as well as reduced socioeconomic development of the countries most affected. The major problem of micronutrient deficiency worldwide is iron deficiency, which is a serious public health problem and a major concern for the World Health Organization (WHO). According to WHO, 30% of the world's population suffers anemia, affecting mainly women and children. Also, the role of iron in fertility and seed yield is an important agronomical trait, because low iron bioavailability in soils, widespread in arable soils, limits plant fertility (Guerinot and Yi, 1994). Recent evidence shows that the amount of micronutrients in seeds has declined systematically since the beginning of the so-called green revolution in the 1960s (Fan et al., 2008; DeFries et al., 2015). Crops biofortification is an agronomic tool that can be implemented to solve malnutrition by increasing the natural content of micronutrients in plants. Biofortification can improve the nutritional content of staple foods that are consumed by the population, resulting in a cost-efficient and sustainable way of delivering nutrients to poor or rural populations with limited access to markets or health centers that provide fortified foods or supplements. To modify the iron content of seeds for human consumption (biofortification) is an attractive alternative to combat iron deficiency, however, mineral loading of seeds is strictly controlled in plants and for instance an increase in root absorption does not produce necessarily seeds with higher



mineral content (Waters and Grusak, 2008; Murgia et al., 2012). Environmental factors and transport activity may be also limiting seed Fe content. Recently, a mechanism depending on ascorbate efflux in plant embryos has been described and it could play a role in the control of iron loading in seeds (Grillet et al., 2014). However, still little is known about transport activity and iron content in seeds. In addition to metabolic and physiological approaches, plant anatomy need to be tackled in order to reach micronutrient improvement in plants (Vasconcelos et al., 2016).

IRON DISTRIBUTION IN Arabidopsis EMBRYO

Elemental imaging of metals using Arabidopsis seeds were previously obtained by X-ray tomography fluorescence (XRF) and Perls/DAB staining; iron accumulates in the vacuoles of the endodermis/perivascular cell layer during *Arabidopsis thaliana* seed maturation (Kim et al., 2006; Roschztardt et al., 2009; Mary et al., 2015). Endodermis cells surround provasculature and iron can be used as a marker of provasculature patterning in Arabidopsis embryos (Roschztardt et al., 2010, 2014). By the time seeds reach maturity the embryo has developed a complete array of procambial strands. After germination, the embryonic provasculature strands differentiate into vascular bundles consisting mostly of phloem and xylem (Sieburth and



Deyholos, 2006). In Arabidopsis, the vein pattern in embryo cotyledons is simple, consisting in primary and secondary veins that can be connected forming areoles (Figure 1). Embryo provasculature cotyledon pattern categories can be defined using Perls/DAB staining, and different degrees of pattern complexity can be found in cotyledons from wild type seeds (Figures 2A,B). Simplest complexity pattern can have only two areoles while four and more areoles are found in the cases of higher complexity (Figures 2A,B). Interestingly, in a wild type seed population, cotyledons with different provasculature complexity patterns are found (Figures 2A, 3). These categories also can be defined in cotyledons by visualizing lignin autofluorescence from mature xylem cells in 1-week-old seedlings (Cnops et al., 2006; Truernit et al., 2012; Roschztardt et al., 2014). Different reports indicate that provasculature vein pattern can be modified either decreasing or increasing its complexity (Carland et al., 2002, 2016; Sieburth et al., 2006; Truernit et al., 2012; Roschztardt et al., 2014). Noteworthy, a change in provasculature complexity does not lead to changes in cotyledon size (Figure 2B). Considering that cotyledon veins are surrounded by the endodermis, *does an increase in the*

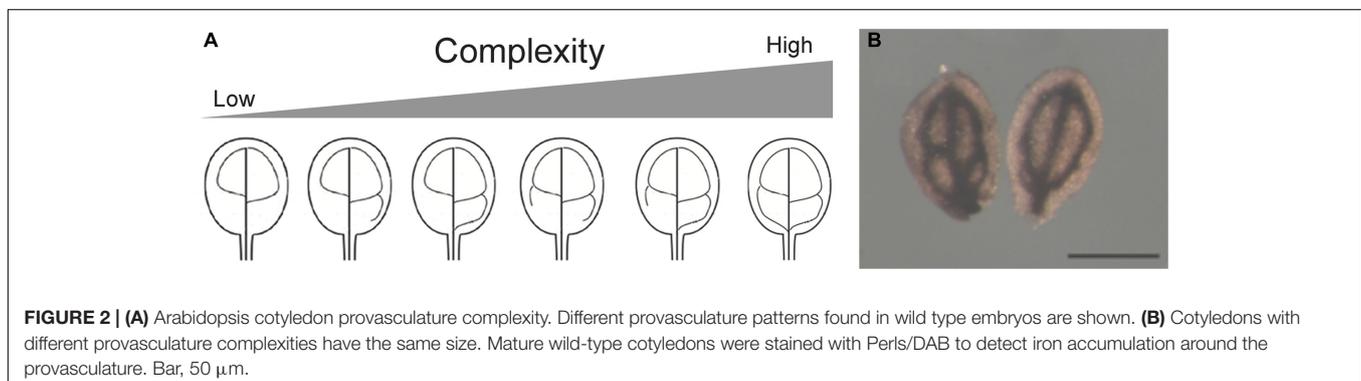


TABLE 1 | Estimation of iron increase in cotyledons with high provasculature complexity.

Vein Complexity ^(a)	Length Ratio of Provasculature ^(b)	Distribution in WT ^(c)	Distribution in HypMut ^(d)	Iron by Complexity WT	Iron by Complexity HypMut
	0.6	0.0	0.0	0.0	0.0
	0.7	4.2	0.0	3.5	0.0
	0.8	4.2	0.0	4.0	0.0
	0.8	16.7	0.0	15.9	0.0
	0.8	43.1	0.0	45.6	0.0
	0.9	23.6	5.0	27.5	5.8
	1.0	2.8	95.0	3.5 100	120.7 126.5
			Total Relative Iron Content ^(e)	100	126.5

^(a) Vein provasculature complexity defined according to Roschzttardt et al. (2014). Category "others" was not included.

^(b) Length ratio of provasculature in cotyledons of 4–1's category.

^(c) Percentage of cotyledons according to Roschzttardt et al. (2014).

^(d) Percentage of cotyledons according to **Figure 3**.

^(e) Only cotyledons.

complexity of the embryo provasculature pattern lead to an increase in Fe content in the seed? In other words, we hypothesize that as consequence of increasing the proportion of cotyledons with four areoles or more, the volume of provasculature and endodermis will also be increased, and therefore iron content will be higher (**Figure 3**). Considering that overaccumulation of free Fe might lead to toxicity by production of reactive oxygen species (Ravet and Pilon, 2013), increasing iron content in seeds through the increase of the number of endodermal cells where iron accumulates in vacuoles can prevent oxidative stress. The mechanisms that control provasculature pattern complexity in Arabidopsis are not well defined, however, it has been described that *OPS* or *VCC* overexpressing plants produce seeds with increased vein pattern complexity in cotyledons (Truernit et al., 2012; Roschzttardt et al., 2014). Overexpression of *OPS* leads to premature phloem differentiation, suggesting that phloem development could positively affect vascular patterning (Truernit et al., 2012). One factor involved in vascular patterning is auxin (Scarpella et al., 2010). However, in *VCC* overexpressing plants, changes in the provascular pattern complexity seem to be not related to auxin transport (Roschzttardt et al., 2014).

In order to address our hypothesis, analysis of Fe content and distribution in different genotypes will be relevant. For instance, seeds from *VCC* and *OPS* overexpressing plants showed an increase of provasculature pattern complexity (Truernit et al., 2012; Roschzttardt et al., 2014) and should be an excellent material to be used as proof of concept. In an opposite way, a decrease in provasculature complexity should lead to the production of seeds with less Fe content. If our hypothesis is correct, seeds from *vcc ops* double mutant plants that have a

less complex provasculature pattern in cotyledons (Roschzttardt et al., 2014), should have a significant decrease in total Fe content. An estimation of the possible iron increasing in cotyledon is shown in **Table 1**. In the case of a distribution of vein complexity like proposed for a hypothetical mutant shown in **Figure 3**, the total iron content could increase around to 26% in cotyledons.

Elucidating the genetic control and signaling involved in the provasculature pattern complexity could be an important step in order to develop strategies to increased iron content in seeds.

CONCLUDING REMARKS

In the Arabidopsis embryo, iron accumulates in the endodermis that surrounds the provasculature. We hypothesize that as an indirect consequence of increasing of provasculature pattern complexity, Fe content in seeds will also increase. This could be used as concept to develop strategies aimed to biofortify seeds.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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