Review

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Iron nutrition, biomass production, and plant product quality

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One of the grand challenges in modern agriculture is increasing biomass production, while improving plant product quality, in a sustainable way. Of the minerals, iron (Fe) plays a major role in this process because it is essential both for plant productivity and for the quality of their products. Fe homeostasis is an important determinant of photosynthetic efficiency in algae and higher plants, and we review here the impact of Fe limitation or excess on the structure and function of the photosynthetic apparatus. We also discuss the agronomic, plant breeding, and transgenic approaches that are used to remediate Fe deficiency of plants on calcareous soils, and suggest ways to increase the Fe content and bioavailability of the edible parts of crops to improve human diet.

Fe is a key determinant of biomass production and of plant product quality

Sustainable intensification is a major challenge for agriculture today [1]. It requires increasing productivity and improving product quality in a sustainable way within the confines of a changing global climate with increasing average CO_2 concentrations and temperatures [2]. Mineral nutrients are major actors in this new scenario. They are essential both for plant productivity and for the quality of their products, and they can affect the environment through the application of fertilizers.

Metals such as Mg, Mn, and Fe are essential because of their role in photosynthetic CO_2 fixation that uses Fe as a key element to ensure electron flow through the PSII–b6f/Rieske–PSI complex (Figure 1). Fe is a limiting factor for biomass production because phytoplankton primary productivity in 30–40% of the oceans is limited by Fe availability [3]. More recently Fe was shown to be a limiting factor for biomass and seed yield in *Arabidopsis (Arabidopsis thaliana)* [4,5] and in crops including tomato (*Solanum lycopersicum*) [6], spinach (*Spinacia oleracea*) [7], and rice (*Oryza sativa*) [8]. In the case of phytoplankton and *Arabidopsis*, the Fe-storage protein ferritin [9] is necessary to buffer transiently the fertilizing Fe in a safe form [4,10], revealing that Fe-dependent biomass production requires the control of Fe homeostasis. Improving plant product quality requires increasing nutrient content and availability. Fe is of primary importance because it is the most commonly deficient micronutrient in the human diet, and Fe deficiency affects an estimated 2 billion people. In this context, Fe biofortification is one possible way to feed humans safely with sufficient Fe directly within their diet [11]. Reaching such a goal requires an integrated knowledge of the establishment and control of Fe homeostasis ([12–16] for recent reviews). We focus here on the emerging outputs of this knowledge that can be implemented in agriculture and biotechnology towards the goal of sustainable production of more nutritious food.

Fe and plant productivity

Fe homeostasis is essential for photosynthesis efficiency

Mesoscale Fe-addition experiments have demonstrated that Fe supply limits biomass production in one-third of the oceans by controlling the dynamics of plankton blooms [17]. Photosynthesis is the engine of this process, and Fe fertilization of the oceans leads to a ninefold increase in the chlorophyll concentration of phytoplankton, which results in doubling their maximum quantum yield of photosynthesis [18].

Fe deficiency is known to alter both chloroplast structure and photosynthetic rate in higher plants [19], but our knowledge of the impact of Fe homeostasis on photosynthesis efficiency, and therefore on biomass production, is still very limited. From a functional point of view, Fe deficiency alters chlorophyll synthesis [20] (Box 1), explaining the interveinal yellowing of leaves known as chlorosis (Figure 2). It also modifies electron transport in both PSI and PSII from dicotyledonous [21,22] and monocotyledonous [23] plants. Adaptation to Fe deficiency involves remodeling of the electron transfer chain (Box 2). The plasticity of the thylakoid membranes in response to Fe deficiency is evidenced by comparative proteome analysis of chloroplast thylakoids [24–26]. These studies report that the protein contents of electron transfer chain components (including the core and light-harvesting components of the PSI and PSII complexes), and of cytochrome *b6/f* (Figure 1), decrease in response to Fe deficiency, with PSI being the most affected. In addition, a transient decrease in trimeric and dimeric organization of the light-harvesting complex (LHC) of PSII from Fe-deficient spinach illustrates an impact on supercomplex organization, a likely adaptive response to energy dissipation in Fe-deficient plants [25]. In rice, probing the PSI core and Lhca1–Lhca4 proteins of LHC1 has been used to assess the

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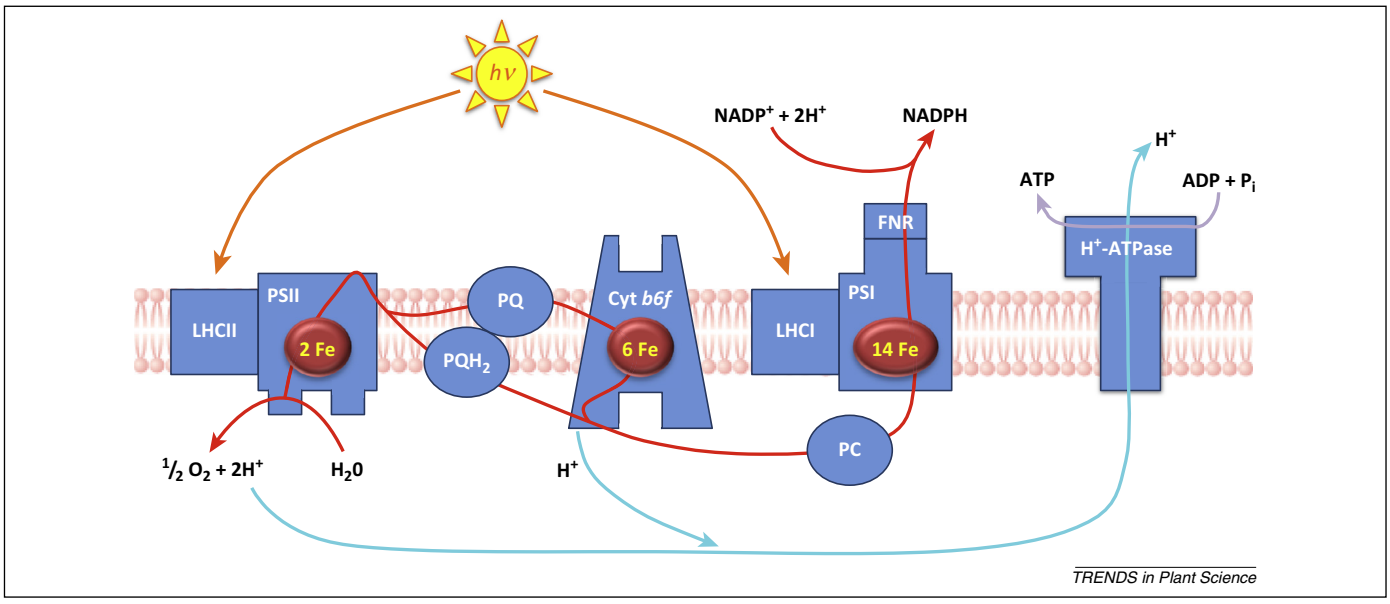

Figure 1. Iron (Fe) is essential for the structure and/or function of the photosynthetic electron transfer chain. Fe is found in the three major complexes of the photosynthetic apparatus (adapted from [19]). Two Fe atoms are present in the photosystem II (PSII) complex: one non-heme Fe is coordinated by four histidines and a bicarbonate, and the second heme Fe is present in cytochrome *b559*. The cytochrome *b6f* (Cyt *b6f*) contains four heme Fe atoms and two Fe atoms in the [2Fe–2S] cluster of the Rieske protein. Three [4Fe–4S] clusters (i.e., 12 Fe atoms) are located in the photosystem I (PSI) complex, and a [2Fe–2S] cluster is found in soluble ferredoxin, and their main function is to carry an electron from the PSI-[Fe–S] complex to the ferredoxin-NADP$^+$ reductase (FNR). The two light-harvesting complexes (LHC) associated with the two photosystems contain chlorophylls, whose synthesis is also Fe-dependent (Box 1). Remodeling of the photosynthetic apparatus according to Fe nutrition conditions has been documented, in particular within the model unicellular green algae *Chlamydomonas reinhardii* (Box 2).

kinetics of PSI subunit degradation in response to Fe deficiency [27]. PsaA and PsaB are stable under Fe deficiency, whereas the levels of PsaC, PsaD, Lhca1, and Lhca2 decrease by 40–50%, and PsaE, Lhca3, and Lhca4 are fully degraded. In addition, several proteins are post-translationally modified; in particular the PSII oxygen-evolving complex is phosphorylated in Fe-deficient plants, whereas it remains unphosphorylated in Fe-replete plants [26]. The biological meaning of such modifications is so far unknown. Furthermore, Fe homeostasis controls the circadian clock rhythm – the mechanism that living organisms employ to adjust their metabolism in anticipation of environmental fluctuations – revealing another key regulatory factor controlling photosynthetic efficiency [28].

Fe nutrition and CO_2 interactions impact upon plant biomass production

Atmospheric CO_2 concentrations have increased by 38% since the start of the industrial era, and could double by the end of the 21st century [29]. About 25% of this CO_2 has been absorbed by the ocean owing to phytoplankton photosynthetic activity that is known to be Fe-dependent [17]. The predictable increase in CO_2 concentration could result in acidification of ocean surface waters, and consequently

Box 1. Fe-dependent cofactors for efficient photosynthesis and plant productivity

Photosynthetic efficiency and the structure and function of the photosynthetic apparatus are heavily Fe-dependent, directly (see Figure 1 in main text) or indirectly via the porphyrin biosynthesis pathway leading to heme and chlorophyll synthesis from their common protoporphyrin IX precursor [83–86]. Furthermore, chloroplasts are autonomous for their Fe–S cluster biogenesis [87,88]. These prosthetic groups are directly involved in the electron transfer chain within the thylakoid membranes where they act as cofactors for various protein complexes [19]. Furthermore, the chlorophyllide *a* oxygenase, involved in chlorophyll *b* synthesis from chlorophyll *a* [84], contains a Rieske Fe–S cluster in addition to a mononuclear Fe center [89]. Upstream of this reaction, the conversion of magnesium (Mg)–protoporphyrin monomethyl ester into divinyl protochlorophyllide is performed by the Mg–protoporphyrin monomethyl ester cyclase, a non-heme carboxylate-bridged di-iron enzyme encoded by the *COPPER RESPONSE DEFECT 1 (CRD1)* or *CHLOROPHYLL 27 (CHL27)* genes in *Chlamydomonas reinhardii* [90] and *Arabidopsis thaliana* [20], respectively. In addition, two Fe-dependent steps occur upstream of the protoporphyrin IX precursor common to both chlorophyll and heme synthesis. Both coproporphyrinogen III oxidase, that is responsible of the conversion of coproporphyrinogen III into protoporphyrinogen IX, and protoporphyrinogen IX oxidase, that is responsible of the conversion of protoporphyrinogen IX into protoporphyrin IX, are likely carboxylate-bridged di-iron enzymes [91], although, to our knowledge, a clear demonstration of this point remains to be established for the plant enzymes. The last step in heme synthesis is the insertion of Fe^{2+} into protoporphyrin IX by the action of the ferrochelatase enzyme. In plants, ferrochelatase is not an Fe protein, in contrast to the [4Fe–4S]-sirohydrochlorin ferrochelatase that is responsible for the synthesis of siroheme, a cofactor of nitrite and sulfite reductases [92].

The chloroplast source of Fe for the synthesis of photosynthesis cofactors is unknown. The potential involvement of the Fe storage protein ferritin in this role is unlikely because its function is to buffer Fe when in excess to avoid oxidative stress [4]. It can explain why the efficiency of Fe fertilization to increase biomass production observed in wild type *Arabidopsis* plants is lost in a *fer1-3-4* triple ferritin mutant. Indeed, the absence of ferritins does not directly affect the photosynthetic electron transfer machinery but does decrease CO_2 fixation, indicating that the photosynthetic electron transfer chain is less efficiently used by the Calvin cycle enzymes in the absence of ferritins [4].