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Article Addendum

Decoding plant responses to iron deficiency

Is nitric oxide a central player?

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Abbreviations: NO, nitric oxide; DNICs, dinitrosyl iron complexes; FRO, Fe^{3+}-chelate reductase; IRT, Fe^{2+}- transporter; GSH, glutathione; MRP1, multidrug resistance-associated transport 1

Key words: iron deficiency, nitric oxide, iron mobilization, dinitrosyl iron complexes, auxin, ethylene

Plants respond to iron deprivation by inducing a series of physiological and morphological responses to counteract the nutrient deficiency. These responses include: (i) the acidification of the extracellular medium, (ii) the reduction of ferric ion and (iii) the increased transport of ferrous ion inside of root cells. This iron transport system is present in strategy I plants and is strictly regulated; at low iron concentration the responses are induced whereas upon iron supply they are repressed. The mechanisms related with this process has been extensively studied, however, the specific cellular effectors involved in sensing iron deficiency, the cascade of components participating in signal transduction, and the way iron is metabolized and delivered, are yet poorly understood. Recently, it has been proposed nitric oxide (NO) as a signaling molecule required for plant responses to iron deficiency. NO is produced rapidly in the root epidermis of tomato plants that are growing under iron deficient conditions. Furthermore, it was demonstrated that NO is required for the expression and activity of iron uptake components in roots during iron deprivation. Here we propose and discuss a working hypothesis to understand the way NO is acting in plants responses to iron deficiency. We specifically highlight the cross talk between NO and plant hormones, and the interaction between NO, iron and glutathione for the formation of dinitrosyl iron complexes (DNICs). Finally, a potential role of DNICs in iron mobilization is proposed.

Iron is an essential mineral nutrient for plant growth and developmental processes.[1] Thereby, plants have evolved generating mechanisms finely controlled to maintain iron homeostasis. Thus, all plants except grasses exposed to iron deficiency induce a set of morphological and physiological responses termed strategy I. These responses are characterized by: (i) induction of rhizosphere acidifiction mediated by the activation of H^+-ATPase, (ii) enhanced activity and expression of Fe^{3+}-chelate reductase (FRO), (iii) increased expression of Fe^{2+}- transporter (IRT) and, (iv) root hair proliferation.[2-5] In addition, it has been identified an essential gene in *Arabidopsis thaliana*, *FIT1*, which encodes a transcription factor that regulates iron uptake responses.[6,7]

Nitric Oxide (NO) is a signal molecule that participates in multiple and diverse physiological responses in plants.[8,9] There are strong evidences that support a previously uncharacterized signalling role for NO mediating iron deficiency responses in roots.[10,11] In a recent report, Graziano and Lamattina[11] have demonstrated that accumulation of *FRO1*, *IRT1* and *FER* (ortholog of Arabidopsis *FIT1*) mRNAs, increased activity of FRO and root hair proliferation are modulated by NO in tomato (*Solanum lycopersicum*) seedlings growing under iron deprived conditions. Furthermore, it has been established that NO production increases in roots of iron deficient tomato seedlings just in the most active region of iron uptake.[11]

It has also been shown that the exogenous application of NO rescued tomato seedlings from the symptoms and phenotype associated to iron deficiency. The NO-induced restoration of normal growth in tomato plants was accompanied by an enhanced expression of responses triggered by the iron deficient conditions.[11] Even though, these results are promising evidence supporting a significant function of NO in plant responses to iron deprivation, no data is available on the molecular basis of the NO targets and action. In addition, there are no reports on the way cells perceive and sense the iron deficiency nor on the nature of signal that communicates iron deficiency between cells. Finally, there are no conclusive experimental data on the way iron is transported and delivered in plants.

NO Plays a Role in the Iron Mobilization and Availability

In animals cells, it is well known that NO forms intracellular complexes which play important functions in biological processes.[12] For instance, S-nitrosothioles and dinitrosyl iron complexes (DNICs) were proposed as the main forms for NO carriers and storage. DNICs are formed by the interaction between NO and Fe^{2+} with thiols-containing ligands. NO releasing agents showed high efficacy in iron mobilization from cells.[12] It has been demonstrated that NO can stimulate cellular iron and glutathione (GSH) release by a mechanism mediated by a multidrug resistance-associated transport 1 (MRP1).[12] Interestingly, electroparamagnetic resonance spectroscopy

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demonstrated that the DNICs peak in NO-treated cells was increased by MRP1 inhibitors, indicating inhibited DNICs transport from cells.[12] These results suggest that NO may mediate iron and GSH efflux from animals cells, by the GSH transporter MRP1, possibly through the formation of DNICs.

However, in comparison, little is known about how the NO participates in iron homeostasis and mobilization in plants. One possibility is that NO promotes iron mobilization as it has already been reported in animals cells.[13] According to this, it has been observed that the levels of GSH enhances in sugar beet roots during iron deficiency[14] and the formation of DNICs increased in China rose leaves treated with gaseous NO.[15] In Arabidopsis, an ATP binding cassette transporter, AtMRP2, has been characterized as a transporter of GSH S-conjugates.[16] Moreover, evidence support that the GSH synthesis is regulated by NO in *Medicago truncatula* roots.[17] Altogether, these results indicate that NO could be mediating an increase in the available iron through DNICs formation. The NO mediated increase of iron mobilization from the roots to leaves, allows them to improve basal metabolism as photosynthesis and reach normal growth conditions even at low iron supply.

NO Regulated Pathways During Iron Deficiency Responses

Auxin and ethylene have been proposed as components of the plant adaptive mechanisms displayed to cope with low iron availability. Arabidopsis mutants defective in auxin or ethylene metabolism as well as experiments made with auxin or ethylene antagonists have demonstrated that these hormones are involved in root hair proliferation during iron deficiency.[18] Auxins are not required, apparently, for the induction of Fe^{3+} chelate reductase activity.[19] Results obtained in our laboratory support NO as an active component operating downstream of auxin in the control of root growth and developmental processes. The auxin-induced formation of adventitious roots, lateral roots and root hair proliferation were promoted by the addition of exogenous NO and were prevented by application of the specific NO scavenger, cPTIO.[20-23] In addition, it was demonstrated that auxin treatment resulted in a localized NO production in roots during lateral and root hair formation.[21,22] Interestingly, iron deficiency induces NO production in tomato[11] and Arabidopsis roots (unpublished results) in a very precise spatial and temporal pattern. Thus, the numerous evidences on NO and auxin participation in root morphology and the NO requirement for plant responses associated to iron deficiency[11] support the hypothesis that NO and auxin would participate in a concerted action to improve the iron bioavailability. Regarding the ethylene role in iron deficiency responses it has been demonstrated that the synthesis of ethylene increases in plants growing in iron deficiency.[24] Ethylene precursors and inhibitors promotes or repress, respectively, the expression and activity of FRO2 and IRT1 of Arabidopsis and FRO1 and IRT1 of tomato plants.[25] Also, it has been proposed that the effect mediated by ethylene on FRO and IRT could be due to variations in FIT or FER levels.[25]

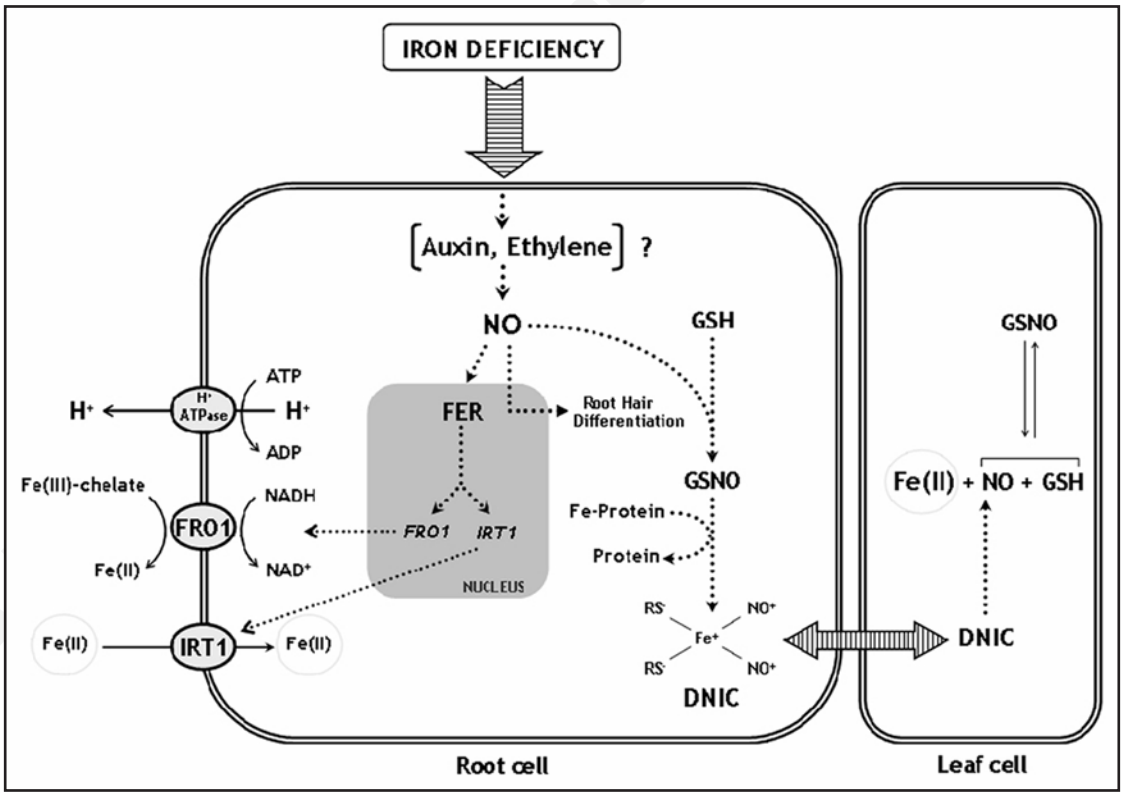

Figure 1. Schematic illustration of a model proposing a role for NO, hormones and DNICs during iron deprivation in strategy I plants. Iron deficiency can activate different pathways, probably via the interrelation between NO and hormones (auxin and/or ethylene). NO production is increased during low iron supply in tomato roots. Increased accumulation of FRO1 and IRT1 transcripts results from an NO mediated enhanced FER expression. In this context, iron uptake system in root cells would be activated. In addition, GSH production induced by iron deprivation together with free or protein associated iron in presence of high NO concentration would induce the formation of DNICs in roots cells. These complexes could be a mechanism of iron transport and delivery to others plant cells or organs improving the bioavailability of iron levels.

Figure 1 shows a simplified scheme representing the participation of hormones, NO, and DNICs in plants responses to iron deficiency. We postulate NO as an active cellular component involved in iron mobilization and availability in plants alleviating symptoms associated to severe iron deficient conditions. It remains to be determined if NO is also involved in sensing and transducing the iron deficiency signal and if the NO function relies on hormonal action. The transduction of signals controlling perception, uptake, metabolism, storage and delivery of iron is a very hot theme in plants and, as consequence, plant biologists are facing a fascinating time of research in plant nutrition.

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