Intra- and intercellular iron trafficking and subcellular compartmentation within roots

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Abstract

Iron is abundant in most soils, but ferric compounds are almost insoluble. Therefore, plant roots use as tools acidification and enzymatic reduction of iron at the outer cell surface (strategy I) or solubilization by phytosiderophores, which are specific ferric chelators (strategy II). In the first case, iron is taken up as Fe2+ into the root symplast, and in the latter one, iron is taken up as Fe(III) complex. The path of iron from the root surface, up to the point of the xylem vessels within the central cylinder, may be completely symplasmic. However, a part of this route also may be an apoplastic one, through the free space of the cell walls of rhizodermis and cortex (apoplast). In the endodermis, the Casparian band forms a strict barrier for apoplastic transport; to move past this site, all ions must enter the symplast. During symplasmic transport, the intracellular environment is protected against the reactive species of iron by handling of iron in chelated forms. A promising candidate for this purpose is the plant-endogenous chelator nicotianamine. At the apoplastic site, iron can be oxidized followed by precipitation as hydroxide or phosphate compounds. Thus, a pool of apoplastic iron can be formed, as shown by reductive mobilization or by proton-induced X-ray emission. This pool may be remobilized when iron deficiency takes place. During radial transport to the vessels, vacuoles may compete with the transport stream forming an iron store. When there is an iron excess, as in plants growing in waterlogged soils or by experimental techniques, plants can escape the deleterious effects of free iron by depositing it in phytoferritin, a storage protein inducible under iron excess. Also, nicotianamine forms a pool of metabolically available iron. Thus, in roots cells of the nicotianamine-free tomato mutant chloronerva iron precipitations occur as evidenced by energy dispersive X-ray analysis and the electron microscopic energy loss technique of energy spectroscopic imaging. Future research concerning the plant root’s iron metabolism are needed to clarify the function of nicotianamine in intra- and intercellular iron trafficking and to identify the so-called iron-sensor which mediates the regulation of iron acquisition reactions of rhizodermal cells in response to the iron nutritional status of the plant.

Abbreviations: EDAX – energy-dispersive X-ray microanalysis; ESI – energy spectroscopic imaging; NMR – nuclear magnetic resonance; PIXE – proton-induced X-ray emission

Introduction

Since Gris (1843) had presented observations on an obvious connection between the supply of plants with soluble ferrous compounds and growth in the French Academy of Sciences, several generations of plant physiologists have explored the function of iron in plant development. Today it is known that iron is an essential component in numerous processes in all plant cells. Therefore, iron nutrition is an important aspect of plant growth and development. However, information concerning acquisition, transport and storage of iron are still limited. Iron is most important because of its physico-chemical properties, which at the same time make it difficult to acquire from soil and to transport it within the plant symplast. Plants require on the order of 10 nM iron in the soil solution for regular growth. However, in calcareous soils the total soluble
iron does not reach values higher than 100 pM. Thus, many plants would suffer from iron deficiency without active mechanisms for extracting iron from the soil. On the other hand, under acidic or reducing soil conditions, e.g., waterlogging, the present excess of Fe(II) would be toxic for plants if they had no instruments to control iron fluxes and to sequester it from the active cell metabolism by specific means of storage.

Root anatomy and transport pathways

Plant roots are more or less permanently growing organs. From that, a sequence follows of different zones from the root tip to the origin of the root. A schematic representation of the different root zones is given for a maize nodal root by Marschner (1995, Fig. 65). The root tip is not able to take up minerals from its environment. It is formed by tissues that function in cell division and differentiation and is supplied with all nutrients by the phloem. Next the root hair zone follows, in which the processes of iron acquisition, uptake, and radial transport to the xylem vessels take place (Marschner et al., 1982).

A schematic cross section of a root shows that two paths are open for iron from the rhizosphere to the xylem vessels (Marschner 1995, Fig. 66). In one, iron can be taken up by rhizodermal cell root hairs into the root symplast after it is reduced (strategy I plants, see below) or after it is bound in a specific complex (strategy II plants). It is then symplastically transported until it is excreted into the xylem. In the other, iron can take the apoplastic pathway through the cell wall network of the cortex, the so-called free space, which amounts to about 5% of the total root volume in maize (Shone and Flood, 1985) and is filled with an aqueous solution of soil minerals and organic compounds such as amino acids, sugars, phenolics and phytosiderophores. This type of transport is concentration-driven diffusion. Particularly in strategy I plants, this gate can be opened and closed by the pH of the apoplastic solution because the solubility product of iron compounds is strongly pH-dependent. So, the calculable value of the Fe$^{3+}$ concentration in the presence of Fe(OH)$_3$ is decreased by a factor of $10^3$ when the pH increases by one unit (Olsen et al., 1981). The Casparian band in the endodermis plays the role of a checkpoint of ion fluxes because of its hydrophobic composition. At this site, all ions must enter the symplast to be transported inside the stele and so the flux rates are controllable for the plant.

For uptake of iron as well as for its excretion into the vessels so-called transfer cells are specialized as an efficient tool to transport ions through membranes (Landsberg, 1986). They are localized in the rhizodermis and in the xylem parenchyma. These cells show numerous cell wall ingrowths, richness of mitochondria and a high density of plasmodesmata. The first property serves as an enlargement of the cell surface to facilitate exchange processes, the second one provides the additional energy required for that purpose.

Iron acquisition and uptake in roots

In many soils, total iron is present at concentrations which are sufficient for the plant’s iron supply. However, because iron is poorly soluble in aerated soils, plants must find ways to acquire iron at a rate that ensures normal plant growth and development. The most powerful tools plants have developed are classified in two strategies (Römheld, 1987). Strategy I, found in dicots and non-graminaceous monocots, consists in the ATPase-driven excretion of protons and the inducible activity of a plasmalemma-bound ferrireductase in the rhizodermis. In this strategy, iron in the rhizosphere is solubilized by acidification and thereafter it is reduced outside the symplast, a prerequisite for its uptake. It then enters the symplast as ferrous ion, probably by a transporter of the IRT1 type which belongs to the ZIP family and has affinities also to other transition metals (Fox and Guerinot, 1998). Under iron deficiency the proton excretion rate as well as the activity of the ferrireductase are markedly increased (Schmidt, 1999).

The strategy II of iron acquisition is restricted to grasses. Their roots release phytosiderophores of the mugineic acid family; these are non-proteinogenic amino acids which act as ferric chelators (Takagi, 1976). They solubilize iron in the rhizosphere by complexation and thereafter these complexes are recognized and transported in toto through the plasma membrane by a yet unknown transport system. It has different affinities to iron complexes of different mugineic acid derivatives. Also, complexes with other micronutrients are accepted, although with significantly lower rates (Ma et al., 1993). When iron becomes scarce, the production and release rates of phytosiderophores are drastically increased. During the increase of their biosynthesis, the first enzyme in this pathway, nicotianamine synthase, plays a key role.
Radial transport across the root

Iron is either taken up in the root symplast of rhizodermal cells or transported apoplasmically and then introduced to the symplast of the cortex. In symplasmic transport, iron has to pass through plasmodesmata that interconnect cells and which are often present in large numbers. For instance, in the endodermis of young barley roots, an average of 20,000 plasmodesmata per cell have been found (Helder and Boerma 1969). During radial transport, a competition may occur between the transport in the symplasm and accumulation of iron in the vacuole. While the Casprian band in the endodermis acts as a selective barrier to control ion fluxes into the symplast of the cortex, it is ruptured in the zone of lateral root formation during penetration of new lateral roots. As consequence, a small portion of iron can enter the stele also apoplasmically by this ‘bypass-flow’.

During symplasmic transport, iron must be shielded by appropriate chelator molecules. Otherwise it would react with reduced forms of oxygen, catalyzing the production of free radicals. These highly reactive oxygen species can damage cellular components and eventually lead to cell death (Guerinot and Yi, 1994). Cells are rich in compounds suitable for iron chelating, especially organic and amino acids. Among these, the non-proteinogenic amino acid nicotianamine seems to have a privileged function (Stephan et al., 1996). As a consequence of iron accumulation in vacuoles of root cells, Pich and coworkers (1997) found an accumulation of the chelator nicotianamine in the vacuoles of cells in the central cylinder using immunhistochemical techniques.

Xylem transport

After radial transport through the symplast into the stele, iron is released into the xylem. This release into the non-living xylem vessels represents a re-transfer from the symplasm into the apoplasm (‘xylem loading’). This release of ions, including iron, is not yet well understood. However, a key role of a respiration-dependent proton pump at the plasma membrane of xylem parenchyma cells is now well established. Protons are pumped into the xylem apoplast (DeBoer et al., 1983) and acidify the xylem sap resulting in a pH lower than 6. This acidification can provide a driving force for cation secretion, through the re-absorption of protons (antiport). Recently, this concept of xylem loading as an energized process has been questioned by Köhler and Raschke (2000). They concluded from their measurements of plasma membrane potential-related fluxes of cations and anions, that the release of ions into the xylem sap occurs through ion channels in a process which is thermodynamically passive.

Driving forces for long-distance xylem flow are a shoot-to-root differential in water potential, or, the generation of root pressure. The first occurs in response to transpiration of shoot tissues, the second can result from water inflow into the xylem after ion release into the vessels; root pressure only contributes to flow when transpiration is low. Within the xylem, according to the fundamental work of Tiffin (1966a, b), iron is seen to be transported as citrate complex in a 1:1 ratio. Under iron deficiency, as part of the metabolic adaptations of root cells, among others, the citrate concentration increases (Brown et al., 1972). The concentration of citrate in the xylem sap simultaneously increases. Although the molar ratio of iron to citrate in the xylem sap can widely differ (López-Millán et al., 2000), citrate remains always in a sufficient excess (Pich et al., 1994).

Phloem transport

The phloem stream serves the root, as iron supply for the growing root tip and as signal transducer for the iron nutritional status of the shoot. Iron can not be transported in the sieve tubes as an ion because the pH in the phloem sap is >7 (Gerendás and Schurr, 1999). This would lead to the precipitation of iron and the transport would be finished immediately after iron had entered the sieve tubes. One must assume that iron is transported in chelated or otherwise protected forms. Since the sum of iron with the other transition metals are loaded in a 1:1 ratio to the chelator nicotianamine, this molecule was favored as iron transporter within the sieve tubes (Stephan et al., 1996;
Von Wirén et al., 1999). But recent findings suggest a protein-bound iron transport, with the protein having high binding affinity in the phloem (Marentes et al., 1997; Wang et al., 1999). Even 1 mM of the reductant sodium dithionite in the presence of the ferrous chelator bathophenanthroline sulfonic acid was not sufficient to release iron from the binding-protein(s) (Schmidtke et al., 1999). Nonetheless, from the co-loading of nicotianamine and the metal micronutrients and from the immunohistochemical distribution pattern of nicotianamine (Krüger and Tiedemann, IPK Gatersleben, personal communication) it is suggested that nicotianamine plays an essential role in phloem loading/unloading processes.

The concentration of iron in the phloem sap, arriving in the tip region of roots, has been argued to be the signal governing the regulation of the iron uptake processes. Maas et al. (1988) demonstrated it using Phaseolus vulgaris plants cultured for 8 days in iron-free nutrient solution. Their roots had acidified the medium and the rhizodermal ferrireductase activity was elevated. At days 3, 6 and 7 the leaves were sprayed with FeEDTA solutions of different concentrations. At day eight, the pH of the medium, reductase activity and the iron concentration of roots were measured. Following iron application to leaves, iron transported to the roots suppressed the iron acquisition mechanisms of the roots as if they were sufficiently iron-supplied, in spite of existing leaf chlorosis; simultaneously, the iron concentration in the phloem sap was measurably increased. But coworkers of the same group could show that the iron efficiency reactions also can be controlled by roots themselves. From potato tubers, which had developed sprouts with roots, the sprouts were excised immediately above the origin of the roots and the roots then were supplied with Knoop’ nutrient solution with or without iron. These sprout-less roots activated all the known strategy I reactions under iron deficiency (Bienfait et al., 1987).

Iron pools and compartmentation

Iron pools can be formed along the route iron is transported. One possibility exists during the process of uptake. On the pathway of iron, from its entry into the root free space (apoplast) to the site of excretion into the xylem vessels, iron pools may be formed. Furthermore, pool formation may occur on the pathway of iron during phloem unloading and symplasmic transport to the cells of the root tip region.

Good prospects for pool formation are sites where iron is not under complete homeostatic supervision, i.e. in the root apoplast. There, the amount of iron is balanced between iron absorption, caused by the more or less activated uptake mechanisms, and iron precipitation, as a result of the physico-chemical conditions occurring in the apoplast. Investigating iron uptake mechanisms in bean roots at supplies in the range of 10 to 500 µM, Jooste and De Bruyn (1979) found the absorption rate to be two- to threefold lower when the 20 min absorption period at 30 °C was followed by a 20 min desorption period with 10 mM Na₂EDTA at 2 °C, compared with experiments without subsequent desorption step. They concluded that iron was precipitated within the tissue (apoplast) which was later solubilized by the chelator. On the other hand, the iron concentrations of tomato roots grown for five days in Hoagland’s nutrient solution with iron concentrations ranging from 0.1 to 100 µM, exceeded many fold those of shoots, although these roots were treated with the desorption procedure of Jooste and De Bruyn for 30 min (Stephan and Grün, 1989). The nature of the high iron contents of roots was analyzed by Bienfait and coworkers (1985) by reductive solubilization, they demonstrated that in roots of bean plants about 75% of the total iron was localized outside the symplast in the free space (apoplast). The size of this pool is determined by the availability of iron, i.e. by the concentration applied in the medium and by the nature of the iron source. This pool is formed by oxidation of ferrous ions, caused by the redox conditions being in the rhizosphere, and subsequent precipitation. It occurs in strategy I- as well as in strategy II plants as demonstrated with bean and maize. Under iron deficiency this pool can be remobilized by rhizosphere acidification and by phytosiderophore excretion (Bienfait et al., 1985; Stephan and Grün, 1989).

The ability of certain genotypes to form larger pools of apoplastic iron than others was correlated with their different chlorosis susceptibility. Longnecker and Welch (1990) could release much more iron from roots of the chlorosis-resistant HA soybean cultivar than from roots of the chlorosis-susceptible PI soybean. When cultivated in iron deficient medium, the HA soybean plants mobilized more iron from their apoplastic pool than the PI soybean plants and chlorosis developed proportionally later in the HA type. However, it must be considered that all results on formation of apoplastic iron pools mentioned
are obtained from plants in hydroponic culture. This may have led to an overestimation of the role of this pool for remobilization under deficiency, especially when compared with soil-grown plants. Recently, this problem was pointed out using the membrane bag technique for roots of different strategy I and II plants, in a PIXE study (Strasser et al., 1999).

There are also intracellular mechanisms to form storage symplastic pools within the root cells in case the iron uptake rate exceeds the cellular demand. Excess uptake may occur in waterlogged soils such as in wetland rice culture. The reason for this is the deficiency of oxygen in the soil followed by a shift of the equilibrium (Fe\(^{3+} \leftrightarrow Fe^{2+}\)) in the direction to ferrous iron. Also, some mutants suffer under iron overload as a result of deregulated iron uptake (see below). A technique known to plant physiologists to cause iron overload is the method of Seckbach (1969). In this procedure, plants are cultured in iron-free nutrient solution until they exhibit a more or less marked chlorosis. Simultaneously, the iron acquisition reactions of the roots are highly activated. Then the plants are transferred for some days into medium containing iron in relatively high concentrations. These plants will take up iron with high rates during the first few days after changing the solution. Thus, they contain more iron than they can cope with. To avoid iron toxicity, the synthesis of the storage protein phytoferritin is induced. It consists of 24 subunits forming a hollow globe with pores for deposition and release of iron. A few thousand atoms of iron can be stored in the central cavity in crystalline form. The chemical nature of this iron deposit is similar to the natural occurring mineral ferrihydrite (Theil, 1987). Most of phytoferritin occurs in plastids (Newcomb, 1967). Phytoferritin can immunologically be detected within 24 h after beginning the Seckbach treatment (Lobréaux et al., 1992).

In the root tip zone a high turnover of iron is necessary to allow intensive growth. It was mentioned above that nicotianamine may play an important role in symplasmic handling of iron. Consequently, it is able to form a pool of ‘active iron’ at sites of high demand or at sites where high iron concentrations transiently emerge. In the first 5 mm region of the root tip of sunflower the nicotianamine concentration is threefold higher than in more basal parts of the root (Stephan and Scholz, 1990). Nicotianamine was immunohistochemically localized in root tip cells basal to the apical meristem of tomato by Pich et al. (1997). The antibodies recognized the free as well as the metal-bound chelator. Label was found to be restricted to vacuoles of cells in the central cylinder. Thus, the iron delivered from the sieve tubes and symptomatically transported in the direction of the meristem, presumably as an iron-nicotianamine complex, may be ‘parked’ in the vacuoles, to be re-called according to demand. A group of peptides, called phytochelatins or class III metallothioneins, are known to bind heavy metals (Grill et al., 1985), but not iron; thus, their impact seems to be restricted to cadmium, copper and zinc (Rauser, 1990). Recently, Dykema and coworkers (1999) reported on a new class of isoprenylated proteins capable of binding transition transition metals; they are presently known, in Arabidopsis, soybean and tobacco. Their isolation procedure suggests that they bind Cu\(^{2+}\), Ni\(^{2+}\) and Zn\(^{2+}\); thus, it is uncertain whether they will also play a role in iron binding, storage or detoxification.

**Mutants affected in iron metabolism**

Several mutants deficient in uptake regulation (for overview see Briat and Lobréaux, 1997) have opened new insights of iron metabolism. Here only three of them will be briefly mentioned. The mutant brz (bronze) of Pisum sativum (L.) cv. ‘Sparkle’ (Kneen et al., 1990) takes up iron with higher rate than it does the wild type. The direct reason for the enhanced iron uptake rate is an elevated Fe(III) reductase activity providing more Fe\(^{2+}\) substrate at the root surface (Grusak et al., 1990). The resulting accumulation of iron in leaves causes for necrotic (bronze) spots by oxidative stress (Welch and LaRue, 1990). The mutation manipulates brz plants to feel functionally as Fe-deficient plants (Grusak et al., 1990). The mutant dgl (degenerated leaflets) of Pisum sativum (L.) cv. ‘Dipples Gelbe Victoria’ (Gottschalk, 1987) hyperaccumulates iron in vegetative organs causing brown degenerative leaves. Irrespective of the extent of iron supply, dgl exhibits ferrireductase activity exceeding that of the wild type. The shoot expression of the dgl gene leads to the generation of a transmissible signal that enhances the reductase activity in roots (Grusak and Pezeshgi, 1996). The mutant chln (chloronerva) of Lycopersicon esculentum (Mill.) cv. ‘Bonner Beste’ (Böhmle and Scholz, 1960) also functionally acts as an iron deficient plant. It permanently exhibits proton extrusion and increased ferrireductase activity (Stephan and Grün, 1989). This mutant is the only known nicotianamine-free plant (Buděšinský et al., 1980) and can be phenotypically normalized by exogenous nico-
tianamine supply. This has opened the door to explore the function of nicotianamine in plant iron metabolism. The permanent acidification of the root apoplast of chln prevents the formation of an apoplastic iron pool. In the root symplast iron is deposited as ferric phosphate precipitates as shown with the EDAX technique (Becker et al., 1995). Such precipitations could even be visualized in root cell mitochondria by the ESI technique (Liu et al., 1998).

Concluding remarks

Some information exists, but much more needs to be understood before we can draw a closed network on iron trafficking and compartmentation in roots. New concepts are emerging concerning the identification of the phloem iron transporter, which imports iron into the root tip region. The possible role of nicotianamine in the loading/unloading processes and symplasmic transport will continue to be clarified. This applies also to the function of the Fe-nicotianamine complex as possible intracellular ‘iron sensor’; this sensor can presumably serve as the trigger to regulate cellular iron metabolism and iron acquisition reactions.

Recently, transgenic plants overexpressing nicotianamine synthase became available, thus, there is a new tool to explore nicotianamine function. The view that iron is predominantly transported as citrate complex in the xylem has been established for some time, but probably should be re-evaluated in additional species. The investigation of the mechanism of iron excretion into the xylem vessels is still completely in its infancy, due mainly to the small flux of iron, relative to macronutrients such as potassium. Once it becomes more sophisticated, the non-invasive NMR technique should serve to further investigate iron fluxes also in roots.

References


