Biochemical Nutrient Pathways in Plants Applied as Foliar Spray: Phosphorus and Iron

VFRC Report 2013/1

Renu Pandey, Vengavasi Krishnapriya and Prem S. Bindraban

VFRC

Biochemical Nutrient Pathways in Plants Applied as Foliar Spray: Phosphorus and Iron

Renu Pandey[1], Vengavasi Krishnapriya[1] and Prem S. Bindraban[2]

[1]Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110012, INDIA
Email: renu_iari@rediffmail.com

[2]Executive Director, Virtual Fertilizer Research Center, Washington, D.C. 20005, USA
Email: pbindraban@vfrc.org

VFRC Report 2013/1

Washington, D.C., USA

Executive Director
Virtual Fertilizer Research Center
1313 H Street NW
11th Floor
Washington, D.C. 20005
USA
E-mail: contact@vfrc.org

Additional information on VFRC can be accessed through http://www.vfrc.org.

Citation

Pandey, R., V. Krishnapriya, and P. S. Bindraban, 2013.

Biochemical nutrient pathways in plants applied as foliar spray: Phosphorus and iron. VFRC Report 2013/1. Virtual Fertilizer Research Center, Washington, D.C. 23 pp.; 6 figs.; 63 ref.

Virtual Fertilizer Research Center

Indian Agricultural Research Institute

Contents

List of acronyms and abbreviations

ABC	ATP-Binding Cassette
ADP	adenine diphosphate
ANT	adenine nucleotide transporter
ATM	ABC Transporter Mitochondria
ATP	adenine triphosphate
bHLH	basic helix-loop-helix
CO_2	carbon dioxide
EDTA	ethylene diamine trichloro acetic acid
ETS	electron transport chain
FCR	Ferric Chelate Reductase
FDR	Fe Deficiency Related
Fe	iron
Fe-S	iron-sulphur
FIT	FER-Like Iron deficiency-induced Transcription factor
FRO	Ferric Reductase Oxidase
H^+	proton
H^+-ATPase	proton-ATPase pump
IRT	Iron Regulated Transporter
MFL	Mito Ferrin-Like
mM	milli Molar
MTP	mitochondrial transport protein
NA	nicotianamine
NADPH	nicotianamine adenine dinucleotide phosphate reduced
NRAMP	Natural Resistance Associated Macrophage Protein
O_2	oxygen
OH^-/Pi	hydroxyl-inorganic phosphate antiport
pH	potential of Hydrogen
P	Phosphorus
Pi	inorganic phosphorus (HPO_4^- / $H2PO_4^{2-}$)
PIC	Permease In Chloroplast
PS	phytosiderophores
PYE	POPEYE
STA	ABC Transporter-like STARK
TCA	tricarboxylic acid
UDP-glucose	uridine diphosphate-glucose
VIT	Vacuolar Iron Transport
YSL	Yellow Stripe-Like
ZIP	ZRT, IRT-like Protein

1 Introduction

In plants, usually it is the root that absorbs water and inorganic nutrients from soil, which are then transported through xylem to the leaf. In leaves, these inorganic nutrient elements are assimilated into various organic compounds by biochemical processes such as photosynthesis and related mechanisms. These organic compounds are then transported from leaf (source) to other plant organs (sink) such as roots, young developing leaves, flowers and fruits. The acquisition of inorganic nutrient elements by roots is interdependent with assimilation activities in leaves. However, root is not the only organ which is involved in uptake of nutrients. The foliage comprising of leaves, stem, inflorescence, and fruits can also absorb nutrient elements. The efficacy of nutrient uptake by foliar-applied spray is governed by the physico-chemical properties of the spray formulation such as pH, surface tension, polarity, spreading and retention of spray fluid. The property of nutrient elements such as molecular size, ionic charge and solubility in spray fluid, in addition to environmental factors like relative humidity, temperature, light and wind affect the rate of foliar uptake. Finally, the plant factors like phenological stage, morphological traits (leaf shape, presence of leaf hairs, stomatal or astomatal, leaf surface architecture, leaf angle and orientation), leaf surface chemistry and mobility of nutrient within the plant system also determines foliar nutrient uptake efficiency (Fernandez and Brown 2013).

The problem with soil fertilization of P and Fe is its bioavailability mostly governed by soil pH. Slow diffusion and high P-fixation in soil leads to lower than 10 µM available P. The P use efficiency is also less with only 10 to 20% being absorbed by plants in the growing season (Werft and Dekkers 1996). Similarly, the availability of Fe is drastically reduced at higher pH range and, being immobile in the plant, its deficiency is more pronounced in growing meristems. Foliar application may be an effective strategy to address the low bioavailability of P and Fe in soil, and for immediate amelioration of nutrient stress at the site of metabolism. Though the soil application of nutrients cannot be completely replaced by foliar application, the latter could definitely boost the plant growth. The purpose of this paper is to review and synthesize the available literature on the general pathways of foliar nutrient absorption and to chalk out the possible biochemical pathways of P and Fe penetration, absorption and their translocation into various cell organelles where they participate in metabolic processes. This would help in identifying effective nutrient composition and developing new-age foliar fertilizers.

2 Entry of foliage applied P and Fe

The major soluble P fertilizers are single superphosphate [$Ca(H_2PO_4)_2$+$CaSO_4$], triple superphosphate [$Ca(H_2PO_4)_2$], monoammonium phosphate [$NH_4H_2PO_4$], diammonium phosphate [$(NH_4)2H_2PO_4$] and phosphoric acid [H_3PO_4] which are being used as foliar spray. Similarly, the commonly available Fe foliar fertilizers are ferrous sulfate, mono- or hepta-hydrate [$FeSO_4.H_2O$], ferric sulfate [$Fe_2(SO_4).4H_2O$] and iron chelate [NaFeEDTA, NaFeHEDTA or NaFeDTPA] (Fageria et al. 2009).

Figure 1 represents the various entry points on leaf and probable pathways in stem, for P and Fe to reach the cell organelle where biochemical reactions take place. The ionized forms of P and Fe are $H_2PO_4^-$ or $HPO_4^{2-}$and Fe^{2+}or Fe^{3+}, respectively. When applied to the foliage, their penetration and absorption into the plant through the various pathways would depend primarily on the ionic charge on plant surface and molecular size.

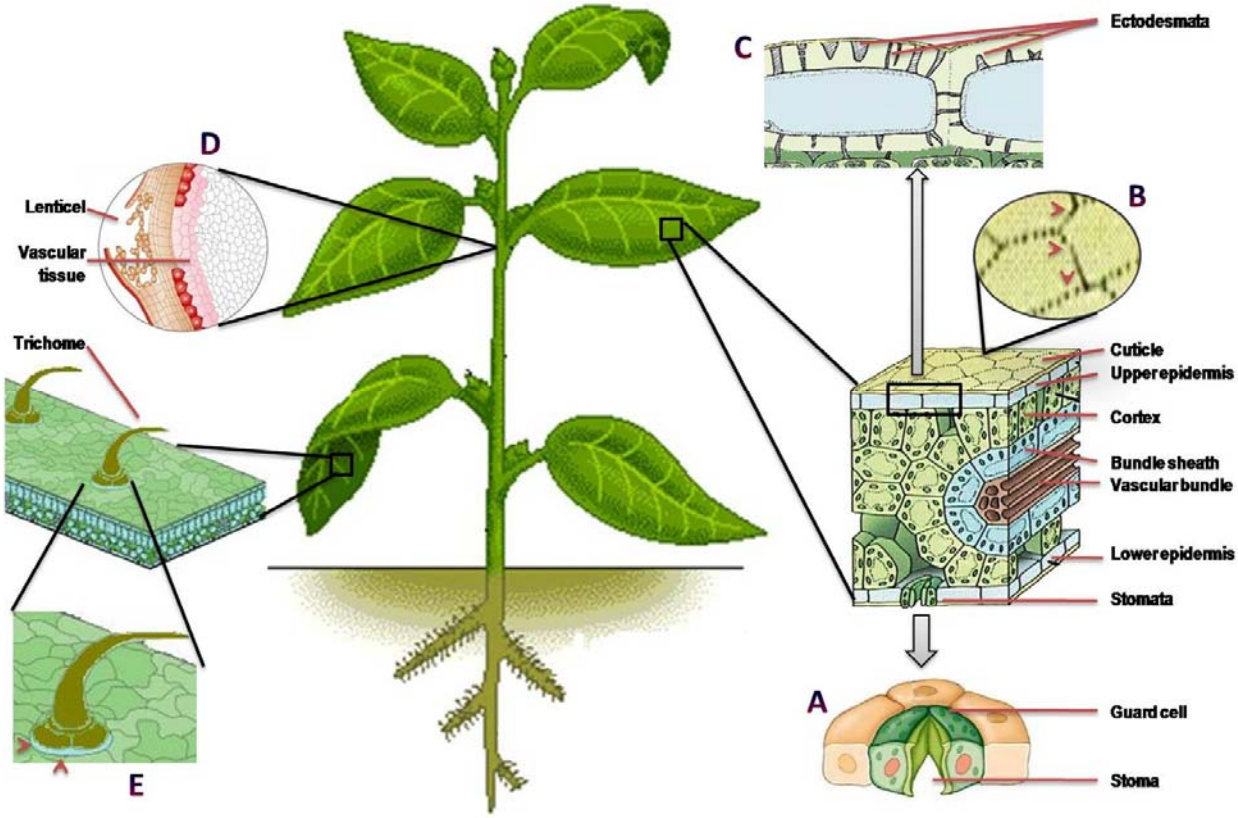

Figure 1. Various points of entry of phosphorus and iron applied as foliar spray.

A: stomata, B: cuticular cracks, C: ectodesmata, D: lenticels, E: aqueous pore.

2.1 Stomata

The upper (adaxial) and lower (abaxial) surface of leaves consists of numerous stomata, an opening for gaseous exchange (CO_2 and O_2) between the internal and external atmosphere of leaf (Figure 1A). The stomatal density is usually higher on the lower surface while a few may be present on the upper surface of leaf. Higher density of cuticular pores in cell walls between the guard and subsidiary cells leads to higher absorption of nutrients (Maier-Maercker 1979). The solutes penetrate through stomata by diffusion along the pore walls, which are less selective and thus offer less resistance as compared to cuticle. Though the overall contribution of stomata to the foliar uptake process is still unclear, but it could serve as a major passage to Fe or P penetration (Figure 2B).

2.2 Cuticular layer

Plant cuticles are lipid-rich protective layers mainly composed of a bipolymer matrix of cutin, waxes, some amount of polysaccharides and phenolics (Yeats and Rose 2013). Presence of cracks on the cuticular surface allows the penetration of solutes (Figure 1B). The different layers of cuticle are shown in Figure 2A. The major resistance to diffusion is offered by the outer layer of the cuticle. This part of the cuticle is called ‘limiting skin’ or ‘limiting layer,’ which amounts to 10% of the cuticle’s total mass. The remainder of the cuticular layer in which the mobility of ions

is greater than in the limiting skin is termed as the 'sorption compartment'. The cuticular penetration of non-charged, lipophilic solutes occurs by dissolution and diffusion while the mechanism of penetration of polar, hydrophilic molecules is not fully understood. Two parallel pathways in cuticles might be responsible for the transport of lipophilic and hydrophilic substances with separate diffusion paths for lipophilic non-electrolytes and hydrated ionic compounds.

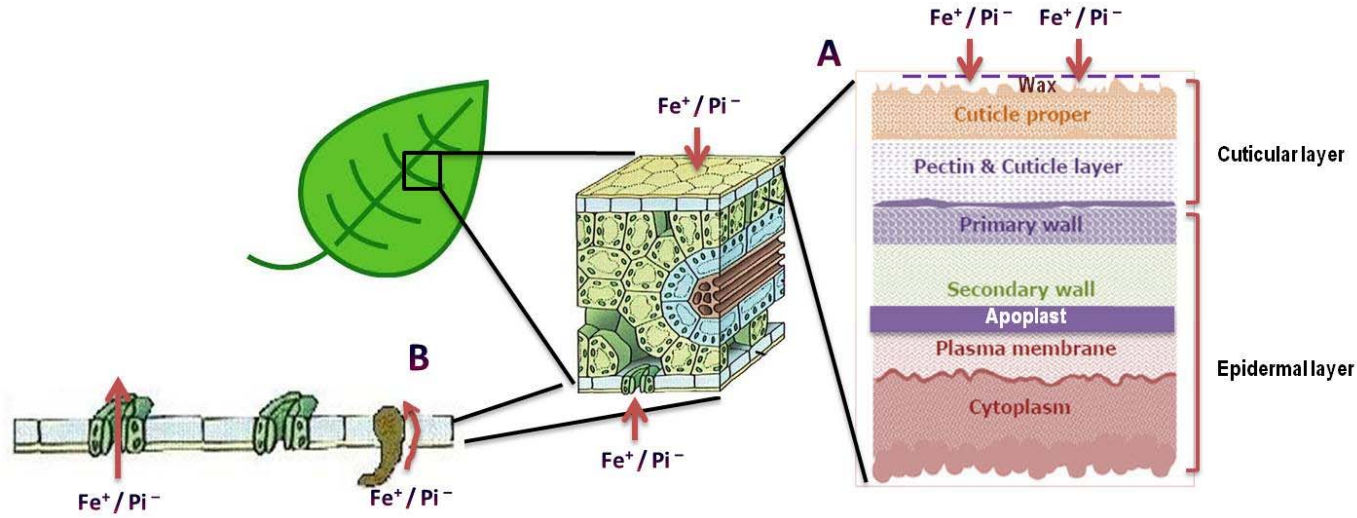

Figure 2. *Penetration of P and Fe through cuticle (A) and stomatal pore (B).*

The outer surface of the cuticle (limiting skin) is negatively charged due to carbonyl/carboxyl group present in cutin. This results in penetration of cations (Fe^{3+}, Fe^{2+}) as they are attracted to the negative charge and diffuse passively. Once the electrical charge is balanced inside the tissue, anions ($H_2PO_4^-$ or HPO_4^{2-}) start to penetrate through the cuticle. The rate of diffusion of ions across the membrane depends on the concentration gradient (Figure 2A). Further, these cuticles are poly-electrolytes and have isoelectric points around 3.0, which means the ion-exchange capacity of the cuticle can be altered by pH fluctuations. So, spray solution with pH values higher than 3.0 will render the cuticle negatively charged, which might allow the diffusion of positively charged Fe while the negatively charged P may get repelled on the surface. Supporting this statement, Komosa (1990) reported that nutrient absorption is higher with pH values between 3.0 and 4.0. However, Zekri and England (2010) stated that the optimum pH of spray solutions should be between 5.0 and 7.5.

2.3 Ectodesmata

Ectodesmata or *teikodes* are well-defined structures in the epidermal wall which terminates on the surface of outer epidermal cell walls (Figure 1C) (Franke 1961, 1967; Michalojc and Szewczuk 2003; Kannan 2010). These structures are usually present on both upper and lower epidermal cells, sides of the larger leaf veins, guard cells, trichomes and epidermal cells surrounding the capitate hairs. Ectodesmata are always covered by the cuticle and do not extend to the outer leaf surface. In this region, the fibrillar structure is comparatively loose than in the cell wall, and the interfibrillar spaces are filled with coarse reticulum of cellulose fibrils, which extends from the plasmalemma to the cuticle. Ectodesmata serves as a polar pathway in absorption and excretion of substances.

In cuticles, the majority of these pores have a diameter of less than 1 nm with a density of about 10^{10}pores cm^{-1}, readily accessible to low molecular weight solutes but larger molecules such as synthetic chelates cannot pass through (Oosterhuis 2009).

2.4 Lenticels

Lenticels are the openings in the bark of stem which are filled by loosely arranged cells called complementary cells (Figure 1D). Lenticels are involved in transpiration; however, their role in absorption of surface applied nutrient solution needs to be ascertained.

2.5 Aqueous pores

Aqueous pores (Figure 1E) are the openings localized in cuticular ledges, preferentially at the basal cells of trichomes, guard cells, and in anticlinal walls. These pores arise due to hydration of permanent dipoles and functional ionic groups (Schonherr 2006). The distribution of polar groups within the lipophilic polymer leads to the formation of isolated aqueous clusters or a continuous aqueous phase if the polar groups are spread out accordingly (Barrie 1968). Average pore radii ranges from 0.45 to 1.18 nm. As the name suggests, aqueous pores are formed only in the presence of water and are dynamic in nature. Since the atomic radius of ionized Fe is 0.126 nm while that of H_xPO_x is 0.157 nm it is very likely that they can penetrate easily through the aqueous pores and reach the epidermal cell walls (Figure 2A).

From the above discussion, it is clear that the polar characteristics of plant surface lead to more efficient translocation of cations (Fe^{3+}, Fe^{2+}) rather than anions (Pi). Further, with the increasing valency, the rate of cation penetration through the cuticle decreases. A charge neutral or apolar molecule (e.g., urea) can pass through the plant surface easily and efficiently. Thus, coating of negatively charged phosphate ions to make it neutral would result in its efficient absorption by plant surface. P combined with Fe such as ferric orthophosphate or ferric phosphate is being frequently used in organic farming as molluscicides but is non-toxic to higher animals. However, ferric phosphate could be one of the potential forms of foliar-applied nutrient that needs experimental confirmation.

3 Biochemical pathways

3.1 Passage of nutrients through the apoplast

Once the nutrient crosses the plant surface and is absorbed, it can take either the apoplastic or symplastic pathway and reach the vascular tissues for its outward translocation. After cuticle, the barriers to nutrient uptake are cell wall and plasma membrane. The cell wall includes primary and secondary cell wall and middle lamella. The cell wall is composed of pectin and polysaccharides, which results in negative charge on the surface due to carboxyl/hydroxyl groups. Therefore, nutrients diffuse across the cell wall against a concentration gradient and reach the apoplast. The characteristics of apoplast are expected to influence the fate of nutrients applied to foliage. In the leaf apoplast, pH value ranges from 4.5 to 7.0 depending on plant species and growing conditions (Yu et al. 2000). The H^+-ATPase located at the plasma membrane pumps H^+ into apoplasm, causing lowering of pH. Apoplast has a role in ionic balance (Grignon and Sentenac 1991) and serves as a transient ion reservoir. It may accumulate cations and repel anions (White and Broadley 2011) and function to adjust the cell turgor (Cram 1999). Leaf apoplast also

functions in detoxification of elements (Sattlemacher 2001), signal transduction leading to stomatal responses (Fujita et al. 2013) and regulation of transient pH fluctuations (Geilfus and Mühling 2011). The ion relation in apoplast varies temporally as a result of changes in metabolic activity caused by day/night transition. Immediately after onset of light, the process of photosynthesis leads to an increase in apoplastic pH (alkalization) as protons are removed from the cytoplasm and apoplast (Mühling and Sattlemacher 1995). The change in apoplastic pH also results due to external factors such as drought, flooding or other stress factors (Felle and Hanstein 2002). It is therefore, expected that the rate of diffusion of Pi or Fe into apoplast would be determined by the pH, and accordingly the application of Pi or Fe compounds should be planned.

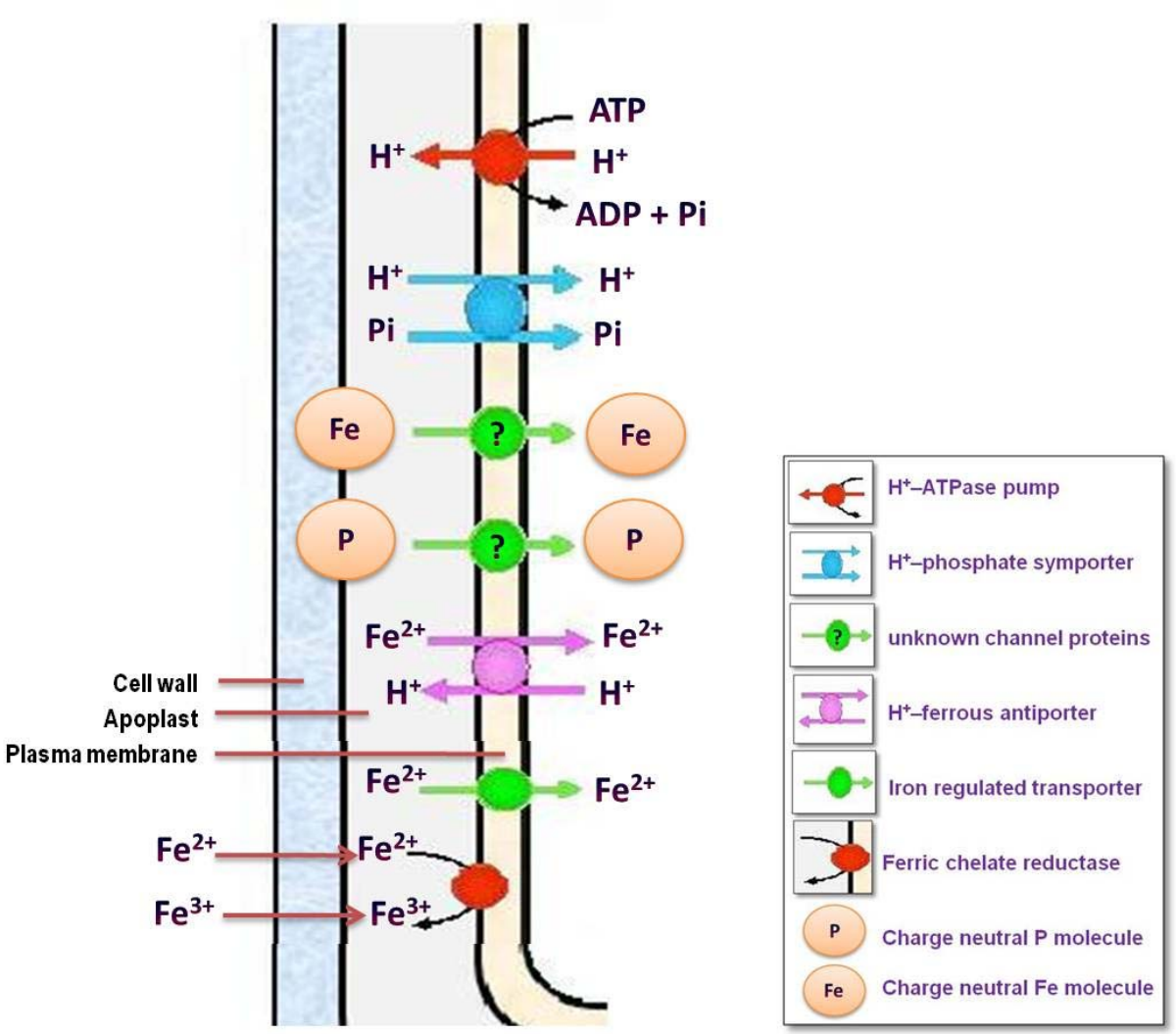

Figure 3. *Transport of P and Fe from apoplast into the cytoplasm through various transporters present in the plasma membrane.*

Circled Pi or Fe denotes charge neutral molecule.

3.2 Phosphorus

The concentration of Pi in a soil solution is usually 1000 times less than that of the root cell due to fixation of P. However, when applied as foliar spray, there is no question of P getting bound with other elements on the leaf surface, resulting in almost 100% absorption of P into the leaf. The absorption of P and its translocation into leaf depends on the wetting agent such as Triton X-100, hygroscopicity of foliar spray (provided it does not form any complex with P) and concentration of P (3.5%) in spray solution (Koontz and Biddulph 1957).

Once the P is absorbed and translocated to the apoplasm of the cell, it moves into the cytoplasm, which takes place through the carrier proteins or transporters present in the plasma membrane. Under optimal P concentration in the growth medium, the Pi concentration in leaf cytoplasm is maintained up to 5 mM (Marschner 1995) by an effective P homeostasis regulated by vacuolar Pi (Mimura et al. 1990). However, the Pi concentration may drop to less than 0.2 mM under P deficiency. For optimal photosynthesis to continue, 2.0 to 2.5 mM P concentration in the cytosol is necessary. In leaf plasma membranes, presence of low-affinity Pi transporters (*Pht2;1*) have been identified, which is a H^+/Pi symporter (Figure 3) and function mainly in loading Pi to shoot organs (Daram et al. 1999). However, if Pi is applied in the form of a charge neutral molecule, it may be translocated into the cytoplasm through non-selective carrier proteins present in the plasma membrane.

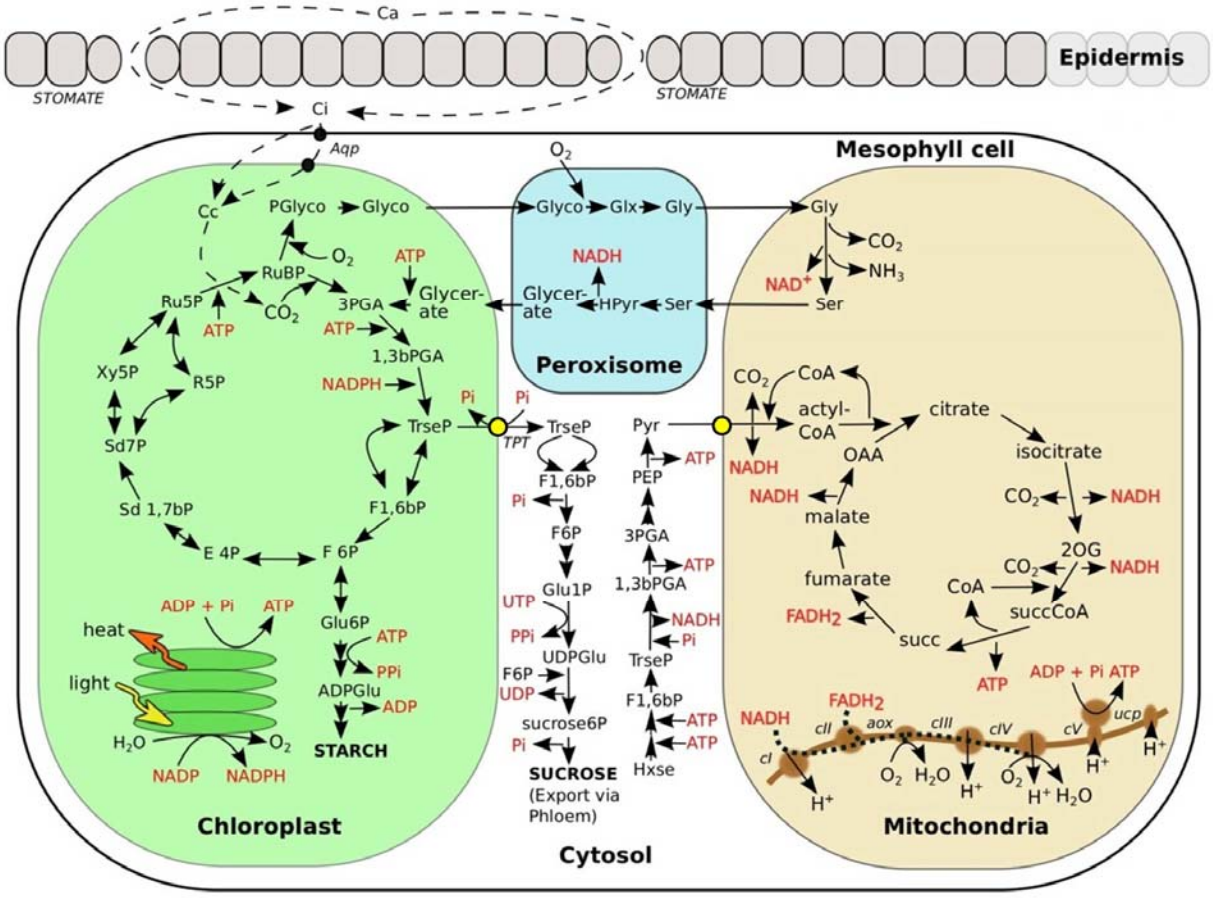

Figure 4. *Metabolic pathways of Pi in the cell.*

In chloroplast, it is involved in the process of photosynthesis generating ATP and Calvin cycle producing starch. In cytosol, Pi is directly involved in sucrose synthesis, while in mitochondria and peroxisome, reducing powers are generated, which pass through the electron transport chain to produce ATP. Pyruvate is transported into the mitochondria *via* a mitochondrial pyruvate carrier (MPC).

Once inside the cytoplasm, Pi is transported to chloroplast through the triose phosphate/Pi translocator present on the chloroplast membrane (Figure. 6), where it is directly involved in the light reaction processes. The photophosphorylation process occurring in thylakoid produces assimilatory (ATP) and reducing power (NADPH) from Pi in the presence of light (Figure. 5). The assimilation of CO_2 through Calvin cycle in chloroplast stroma producing starch involves synthesis of various sugar-phosphate intermediary compounds (Figure. 4). In cytoplasm, Pi is involved in synthesis of sucrose *via* UDP-glucose which is then exported to other developing organs through phloem. The loading of sucrose into phloem at 'source' and unloading at 'sink' against a concentration gradient is an active process and requires ATP. The Pi is also involved in producing ATP inside the mitochondria through TCA cycle and ETS. Entry of pyruvate and Pi into mitochondrial matrix takes place by OH^-/Pi antiport through MTPs located on the inner membrane (Figure. 6). An adenine nucleotide transporter (ANT) is involved in facilitating the exchange of ADP^{3-} (inward) for ATP^{4-} (outward). However, the small, neutral and hydrophobic molecule can also diffuse into mitochondria, but the pore-forming protein in the outer membrane of mitochondria (porins) needs further characterization.

3.3 Iron

Iron plays a crucial role in metabolic processes as it is a metal cofactor of many enzymes which carry out oxidation-reduction reactions involving photosynthetic electron transport, nitrate reduction and assimilation, chlorophyll biosynthesis, all requiring Fe as Fe-heme group or Fe-S cluster. Few data are available on how Fe enters the leaf cell. When applied to the foliage, Fe diffuses through the cuticular layers and cell membranes, and enters the apoplasm as described above. If the leaf apoplastic pH is higher, it will immobilize Fe (Kosegarten et al. 1999) thus causing inhibition of metabolic processes which require Fe, and finally leading to Fe deficiency symptoms. From apoplast, Fe is transported into the cytosol through plasma membrane.

The mechanism of Fe uptake in plants *via* roots is based on two strategies: Strategy I involves reduction of Fe^{3+} to Fe^{2+} carried out by membrane bound enzyme FCR. The reduced form of iron is then taken up by the metal transporter called IRT into the cell. This mechanism operates in dicotyledonous plants. Strategy II, a chelation based mechanism occurs in Poaceae (grass) family where roots release PS which forms complex with Fe^{3+} and is taken up by plant as Fe^{3+}-PS chelate (Romheld and Marschner 1986). Similar to roots, iron uptake in leaf mesophyll cells is also mediated by reduction process carried out by FCR (Figure. 3). However, the presence of IRT in the leaf has not been characterized. The genes encoding FCR enzyme belongs to the FRO family, and the IRT belongs to ZIP family (Wu et al. 2005, Mukherjee et al. 2006, Jeong et al. 2008, Guerinot 2000). In Arabidopsis, *FRO2* gene identified in root has been found to encode for Fe^{3+}-chelate reductase (Mukherjee et al. 2006). The root Fe uptake mechanism is tightly regulated by a complex system involving transcription factors, bHLH, of which PYE and FIT/FER play a central role (Ivanov et al. 2012).

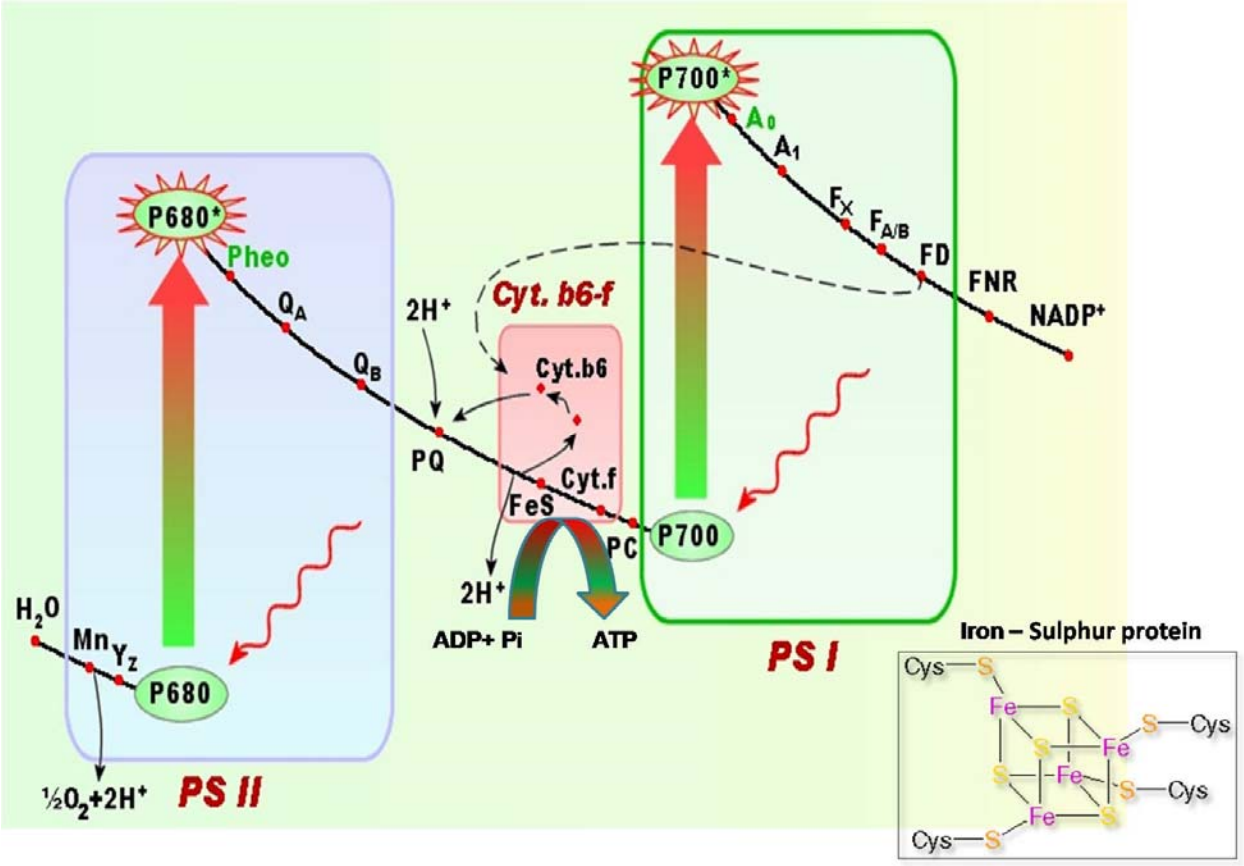

Figure 5. *Involvement of Pi and Fe in photophosphorylation in thylakoid membrane of chloroplast.*

Pi is involved as ATP, while Fe is a constituent of Fe-S (iron-sulphur protein), cytochrome f and cytochrome b6; and a cofactor of ferredoxin.

FCR system was first reported in mesophyll cells of *Vigna unguiculata* leaf (Brüggemann et al. 1993) and was later confirmed in sunflower leaf disks (De la Guardia and Alcántara 1996) which reduced Fe^{3+}-EDTA and Fe^{3+}-citrate. The natural complexes of Fe^{3+} with citrate and malate were found to be good substrates for the FCR enzyme present in leaf plasma membrane. The optimal pH for the activity of FCR enzyme in leaf plasma membrane was reported to be in the range 6.5–7.0 suggesting that changes in apoplastic pH could significantly decrease the activity (González-Vallejo et al. 1999). The affinity of FCR enzyme for substrate was in the order Fe^{3+}-malate > Fe^{3+}-EDTA > Fe^{3+}-citrate, with apparent K_m values of 73, 122 and 157 for Fe-deficient leaves (Brüggemann et al. 1993) as that for plasma membrane of roots (Susín et al. 1996). Unlike root, the FCR activity in leaf is not enhanced by Fe deficiency due to an insufficient reducing power (NADPH) in Fe deficient cells (González-Vallejo et al. 2000). The identity of the physiological substrates for this enzyme is still an open question. Photo-reduction of Fe^{3+}-citrate complexes in leaf apoplast by light has also been proposed that would facilitate uptake of Fe^{2+} into the leaf cell symplast by IRT transporters (Nikolic et al. 2007).

Besides IRT, other transporters may also be involved in Fe uptake by leaf cells, which includes several members of the YSL family that are expressed in leaves (such as AtYSL1, AtYSL3 and AtYSL2 in *Arabidopsis thaliana*,

OsYSL2 and OsYSL15 in rice), but their expression is usually confined to the vascular tissue (Curie et al. 2009). The specificity of some of these transporters with the possible substrates is still not known. Fe can also take the symplastic route with plasmodesmata facilitating extensive communication between leaf cells.

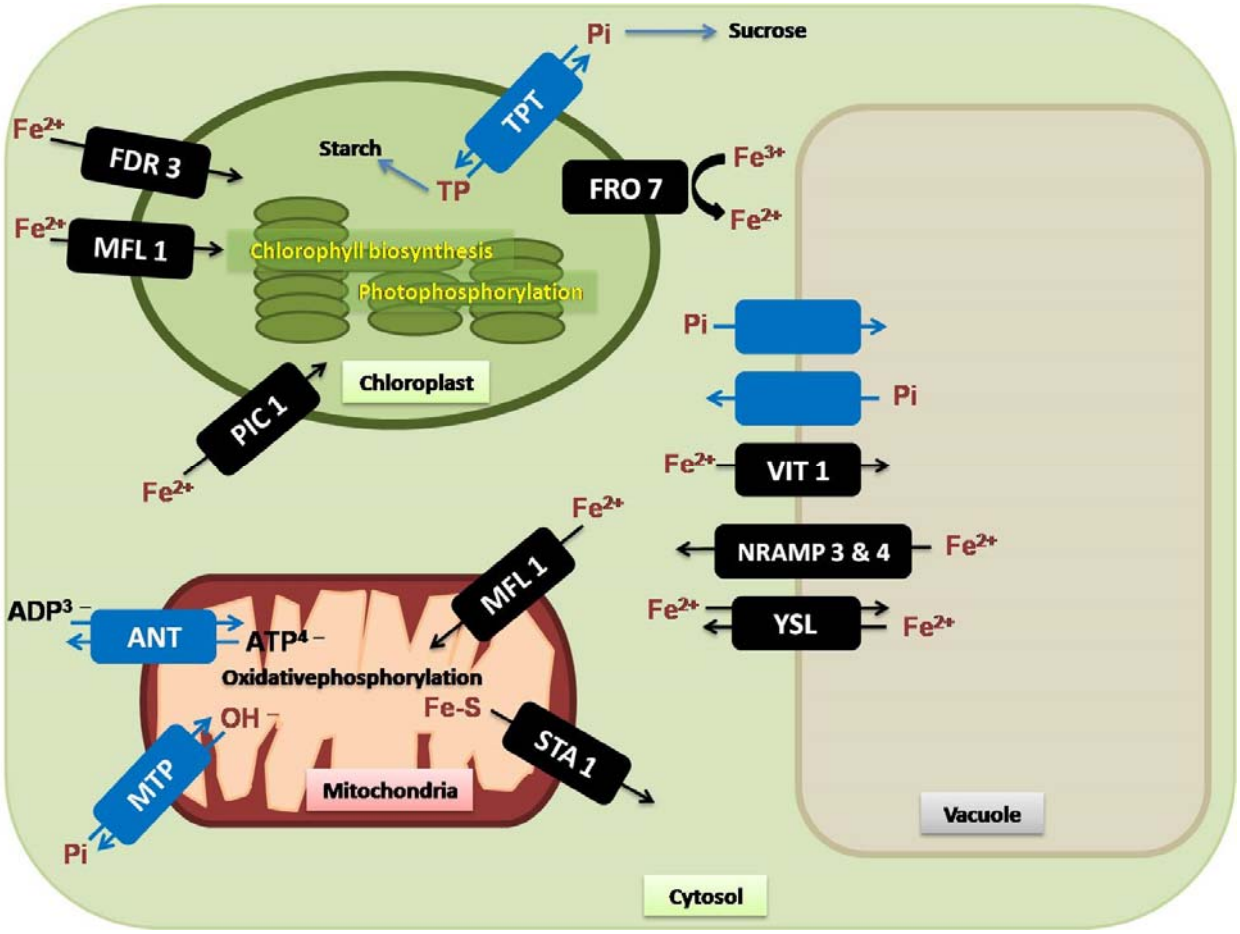

Figure 6. *Translocation of Fe and P from cytosol into the chloroplast, mitochondria and vacuole.*

Entry of Fe into chloroplast takes place through PIC 1 (Permease in Chloroplast), MFL 1 (Mito Ferrin Like) and FDR 3 (Fe Deficiency Related); while FRO 7 (Ferric-chelate Reductase Oxidase) reduces ferric to ferrous form. VIT 1 (Vacuolar Iron Transport) imports Fe into vacuole. NRAMP 3 and NRAMP 4 (Natural Resistance Associated Macrophage Protein) proteins are involved in Fe export from vacuole, while YSL (Yellow Stripe Like) performs dual function. Fe enters the mitochondria through MFL 1, forms Fe-S centers and, exported to the cytosol through STA 1 (ABC-type transporter STARIK). Pi enters chloroplast via TPT (Triose phosphate/inorganic Phosphate antiporter). Mitochondrial transport proteins (MTP) are involved in influx of Pi, whereas Pi as a constituent of ATP (adenosine triphosphate) enters the mitochondria through ANT (adenine nucleotide transporter) protein. Vacuolar fluxes of Pi are mediated through inorganic phosphate transporters.

Once the Fe^{2+} enters the cytoplasm through plasma membrane, it is involved in metabolic processes either in the cytoplasm or enters organelles such as mitochondria, chloroplast or vacuole. Although mitochondria contain Fe-S proteins but the mechanism of iron influx in mitochondria is not well understood. The Fe-S cluster participates in oxidative phosphorylation to produce reducing powers. Efflux of Fe from mitochondria by an ABC type transporter

protein known as STA1, has been identified in Arabidopsis (Kushnir et al. 2001) which is a homologue of yeast ATM1 protein located at the inner mitochondrial membrane (Figure 6). Another gene encoding MFL1 transporter has been reported from Arabidopsis responsible for Fe transport into chloroplasts under various conditions of Fe supply. It was found that if the expression of AtMFL1 is suppressed, the plant Fe accumulation in various developmental stages is altered (Tarantino et al. 2011).

Excess of iron is stored in the vacuole through various transporters (Figure 6) which is remobilized during seedling development. The transporter involved in Fe influx has been characterized as VIT1 while for efflux, NRAMP3 and NRAMP4 transporters have been identified in Arabidopsis (Lanquar et al. 2005; Kim et al. 2006). Recently, NRAMP5 identified in plasma membrane of root cells as well as in pistil, ovary, lemma and palea of rice is constitutively expressed and play a role in Fe and Mn transport during flowering and seed development (Ishimaru et al. 2012). Besides NRAMP family, YSL4 and YSL6 transporters identified from Arabidopsis vacuole are expected to act in both influx and efflux of Fe from vacuole (Jaquinod et al. 2007).

The transport of Fe into chloroplast takes place *via* PIC1 as identified in Arabidopsis (Duy et al. 2007, 2011). In chloroplast membrane, FCR enzyme is located belonging to FRO7 catalysing Fe^{3+} to Fe^{2+} in the presence of light (Bughio et al. 1997) (Figure. 6). Localization of another Fe transporter FDR3 by fluorescence immunocytochemistry in plastids of root, stem and leaves of maize was reported which carried out Fe influx (Han et al. 2009). Fe plays a key role in chloroplast as it is involved in chlorophyll biosynthesis as well as electron flow through Fe-S cluster protein, and excess of Fe is stored as Fe-ferritin protein (Roschzttardtz et al. 2013). Besides these organelles, high concentration of Fe has also been found in pea (*Pisum sativum*) nucleus where it is accumulated in a sub-compartment called the nucleolus (Roschzttardtz et al. 2011). However, the mechanism of Fe transport into nucleus has not been well understood.

The long-distance iron transport takes place through xylem and phloem. The transporter that loads Fe into the xylem is not yet identified but it has been reported that in cytosol, at neutral pH, Fe is chelated with the non-proteinogenic amino acid, NA (von Wirén et al. 1999, Rellán-Álvarez et al. 2008). Nicotianamine is expected to play major roles in symplastic and phloem Fe chelation. This Fe^{3+}-NA complex is loaded into the xylem by a Fe^{3+}-PS transporter from the YSL family. The rice ortholog OsYSL2 has been found to be localized in the lateral plasma membrane and transports Fe^{3+}-NA and is proposed to be involved in phloem transport (Koike et al. 2004). However, the knowledge on Fe xylem loading is still scarce and many questions regarding chemical form(s) and mechanism(s) involved in Fe loading are still open.

4 Conclusions

Although the penetration/absorption of foliar-applied nutrients has been experimentally proved, some of the entry points, such as lenticels, need further study. Absorption of nutrients is followed by translocation to the sites of metabolic processes. The literature survey revealed that if the nutrient molecule is made charge neutral, there is possibility that it will pass through the plasma membrane.

Photosynthesis is the primary process in which CO_2 is fixed in the presence of light, which is biochemically converted to carbohydrates. In the process, ATPs are produced which are the energy molecule required in all other

anabolic processes such as synthesis of lipids, protein, nucleic acids, vitamins, etc. Since the Pi molecule is one of the components of ATP, it is directly involved in all metabolic processes. In physiologically mature grains, the forms of P and Fe stored in the edible parts of plants are phytate and ferritin, respectively. P is stored in seeds or bran as phytate or phytic acid (inositol-hex*akis*phosphate; IP6), a saturated cyclic acid. The excess Pi is stored in vacuole in the form of phosphate monoesters and polyphosphate which is remobilized under the conditions of Pi-stress and helps in plant growth. Further, there is no difference in the biochemical inclusion of Pi or Fe into the metabolic processes in plants on their source, whether it is absorbed by roots or leaves.

Agronomic biofortification is one of the approaches to increase the grain nutrient content by application of nutrients as foliar spray during grain growth period. However, nutrient concentration in excess of plants' demand (Pi >1%; Fe >0.03%) causes toxicity and interferes with mobility of other elements in the plants (e.g., Pi interferes with zinc and copper; Fe with calcium).

5 Recommendations requiring further research/testing

- For iron absorption through foliage, there is possibility to chelate the Fe^{3+} with artificially synthesized mugineic acid (nicotianamine), which otherwise is released by roots under Fe deficiency. Since the leaf cells possess an FRC enzyme system to reduce the Fe^{3+}, delivery of chelated Fe^{3+}-NA is possible.
- The channel protein 'porins' needs to be identified and characterized since it can allow passage of charge neutral molecules such as Fe^{3+}-NA complexes.
- If Pi is made charge neutral, it is still not known how the 'neutral' Pi molecule will dissociate itself from the carrier once it is inside the cytosol since no such enzyme system has been reported. However, as mentioned previously, ferric phosphate could be one of the potential forms of foliar-applied nutrient, but experimental confirmation is warranted.
- Further, from the literature it is clear that, several transport proteins in leaf cell membranes have been identified and characterized which are involved in translocation of Fe and Pi. But this work is still at its initial stage as mostly they are characterized in the model plant Arabidopsis. Therefore, these transporters need to be characterized in crop plants.
- High-affinity Pi and Fe transporters are expressed exclusively in roots and induced by low concentration of these elements. However, no literature is available whether such transporters when expressed in leaves using leaf-specific promoters will lead to enhanced nutrient absorption. This might provide an answer to whether the uptake of foliar applied fertilizers could be enhanced.
- Injecting nutrients directly into the vascular tissue would be synonymous to nutrient absorption through lenticels present in the stem. Lenticels are the openings in the stem and are nothing but the phloem, so nutrients absorbed through lenticels can move *via* phloem and thus be translocated to both root and shoot. Nutrients in xylem can be translocated to phloem through 'transfer cells', however, whether the reverse process also occurs is not well understood.

6 Acknowledgements

The authors RP and VK acknowledge the Indian Agricultural Research Institute and Indian Council of Agricultural Research, New Delhi, India, for providing infrastructural facility and Drs. Viswanathan C. and Sangeeta Khetarpal for their valuable discussions on the subject. This work was supported by funding from the Virtual Fertilizer Research Center, Washington, D.C., USA.

7 References

Barrie, J.A., 1968.

Water in polymers. In: Crank J., Park G.S., eds. *Diffusion in Polymers*. London: Academic Press, 259–314.

Bughio, N., M. Takahashi, E. Yoshimura, N.K. Nishizawa and S. Mori, 1997.

Light-dependent iron transport into isolated barley chloroplasts. *Plant Cell and Physiology*, 38, 101–105.

Bruggemann, W., K. Maas-Kantel, and P.R. Moog, 1993.

Iron uptake by leaf mesophyll cells: The role of the plasma membrane-bound ferric-chelate reductase. *Planta*, 190, 151–155.

Cram, W.J., 1999.

Negative feedback regulation of transporting cells. The maintenance of turgor, volume and nutrient supply. In: U. Lüttge, M.G. Pitman, eds. *Encyclopedia of Plant Physiology*. Germany: Springer, 284–316.

Curie, C., G. Cassin, D. Couch, F. Divol, K. Higuchi, M. Le Jean, J. Misson, A. Schikora, P. Czernic, and S. Mari, 2009.

Metal movement within the plant: Contribution of nicotianamine and yellow stripe 1-like transporters. *Annals of Botany*, 103, 1–11.

Daram, P., S. Brunner, C. Rausch, C. Steiner, N. Amrhein, and M. Bucher, 1999.

Pht2;1 encodes a low-affinity phosphate transporter from Arabidopsis. *The Plant Cell*, 11, 2153–2166.

De la Guardia, M.D. and E. Alcántara, 1996.

Ferric chelate reduction by sunflower (*Helianthus annus L.)* leaves: influence of light, oxygen, iron deficiency and leaf age. *Journal of Experimental Botany*, 47, 669–675.

Duy, D., R. Stube, G. Wanner, and K Philippar, 2011.

The chloroplast permease PIC1 regulates plant growth and development by directing homeostasis and transport of iron. *Plant Physiology*, 155, 1709–1722.

Duy, D., G. Wanner, A.R. Meda, N. von Wiren, J Soll, and K. Philippar, 2007.

PIC1, an ancient permease in Arabidopsis chloroplasts, mediates iron transport. *Plant Cell*, 19, 986–1006.

Fageria, N.K., M.P. Barbosa Filho, A. Moreira, and C.M. Guimarães, 2009.

Foliar fertilization of crop plants. *Journal of Plant Nutrition*, 32, 1044–1064.

Fernández, V. and H.B. Patrick, 2013.

From plant surface to plant metabolism: The uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science*, 4, 1–5.

Felle, H.H. and S. Hanstein, 2002.

The apoplastic pH of the substomatal cavity of the *Vicia faba* leaves and its regulation responding to different stress factors. *Journal of Experimental Botany*, 53, 73–82.

Franke, W., 1961.

Ectodesmata and foliar absorption. *American Journal of Botany*, 48, 683–691.

---------, 1967.

Mechanism of foliar penetration of solutions. *Annual Review of Plant Physiology*, 18, 281–301.

Fujita, T. K. Noguchi, and I. Terashima, 2013.

Apoplastic mesophyll signals induce rapid stomatal responses to CO_2 in *Commelina communis*. *New Phytologist*, 199, 395–406.

Geilfus, C.M. and K.H. Muhling, 2011.

Real-time imaging of leaf apoplastic pH dynamics in response to NaCl stress. Frontiers in Plant Science, 2, 13.

Gonzalez-Vallejo, E.B., J.A. Gonzalez-Reyes, A. Abadia, A.F. Lopez-Millan, F. Yunta, J.J. Lucena, and J. Abadia, 1999.
Reduction of ferric chelates by leaf plasma membrane preparations from Fe-deficient and Fe-sufficient sugar beet. *Australian Journal of Plant Physiology*, 26, 601–611.

González-Vallejo, E.B., F. Morales, L. Cistué, A. Abadía, and J. Abadía, 2000.
Iron deficiency decreases the Fe (III)-reducing activity of sugar beet protoplasts. *Plant Physiology*, 122, 1–8.

Grignon, C. and H. Sentenac, 1991.
pH and ionic conditions in the apoplast. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42, 103–128.

Guerinot, M.L., 2000.
The ZIP family of metal transporters. *Biochimica et Biophysica Acta – Biomembranes*, 1465, 190–198.

Han, J.H., X.F. Song, P. Li, H.J. Yang, and L.P. Yin, 2009.
Maize ZmFDR3 localized in chloroplasts is involved in iron transport. *Science in China Series C: Life Sciences*, 52, 864–871.

Ishimaru, Y., K. Bashir, H. Nakanishi, and N.K. Nishizawa, 2012.
OsNRAMP5, a major player for constitutive iron and manganese uptake in rice. *Plant Signaling Behaviour*, 7, 763–766.

Ivanov, R., T. Brumbarova, and P. Bauer, 2012.
Fitting into the harsh reality: regulation of iron-deficiency responses in dicotyledonous plants. *Molecular Plant*, 5, 27–42.

Jaquinod, M., F. Villiers, S. Kieffer-Jaquinod, V. Hugouvieux, C. Bruley, J. Garin, and J. Bourguignon, 2007.
A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. *Molecular Cell Proteomics*, 6, 394–412.

Jeong, J., C. Cohu, L. Kerkeb, M. Pilon, E.L. Connolly, and M.L. Guerinot, 2008.
Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions. *Proceedings of the National Academy of Sciences USA*, 105, 10619–10624.

Kannan, S., 2010.
Foliar fertilization for sustainable crop production. *Sustainable Agricultural Revolution*, 4, 371–402.

Kim, S.A., T. Punshon, A. Lanzirotti, A. Li, J.M. Alonso, J.R. Ecker, J. Kaplan, and M.L. Guerinot, 2006.
Localization of iron in Arabidopsis seed requires the vacuolar membrane transporter VIT1. Science, 314, 1295–1298.

Koike, S., H. Inoue, D. Mizuno, M. Takahashi, H. Nakanishi, S. Mori, and N.K. Nishizawa, 2004.
OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *Plant Journal*, 39, 415–424.

Koontz, H. and O. Biddulph, 1957.
Factors affecting absorption and translocation of foliar applied phosphorus. *Plant Physiology*, 32, 463-470.

Komosa, A., 1990.
Influence of selected chemical properties of solutions and nutritional status of plants on the efficiency of foliar nutrition of greenhouse tomato. Dissertation, *Scientific Papers of the Agricultural University of Poznan*, p120.

Kosegarten, H.U., B. Hoffmann, and K. Mengel, 1999.
Apoplastic pH and Fe3+ reduction in intact sunflower leaves. *Plant Physiology*, 121, 1069–1079.

Kushnir, S., E. Babiychuk, S. Storozhenko, M.W. Davey, J. Papenbrock, R. De Rycke, G. Engler, U.W. Stephan, H. Lange, G. Kispal, R. Lill, and M. Van Montagu, 2001.
A mutation of the mitochondrial ABC transporter STA1 leads to dwarfism and chlorosis in the Arabidopsis mutant STARIK. *Plant Cell*, 13, 89–100.

Lanquar, V., F. Lelièvre, S. Bolte, C. Hamès, C. Alcon, D. Neumann, G. Vansuyt, C. Curie, A. Schröder, U. Krämer, H. Barbier-Brygoo, and S. Thomine, 2005.
Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *EMBO Journal*, 24, 4041–4051.

Maier-Maercker, U., 1979.
Peristomatal transpiration and stomatal movement: a controversial view. III. Visible effects of peristomatal transpiration on the epidermis. *Zeitschrift fur Pflanzenphysiologie*, 91, 225–238.

Marschner, H., 1995.
Mineral Nutrition of Higher Plants, 2nd ed. London: Academic Press.

Michalojc, Z. and C. Szewczuk, 2003.
Theoretical aspects of foliar nutrition. *Acta Agrophysica*, 85, 9–17.

Mimura, T., K.-J. Dietz, W. Kaiser, M.J. Schramm, G. Kaiser, and U. Heber, 1990.
Phosphate transport across biomembranes and cytosolic phosphate homeostasis in barley leaves. *Planta*, 180, 139–146.

Mühling, K.H. and B. Sattelmacher, 1995.
Apoplastic ion concentration of intact leaves of field bean (*Vicia faba*) as influenced by ammonium and nitrate nutrition. *Journal of Plant Physiology*, 147, 81–86.

Mukherjee, I., N.H. Campbel, J.S. Ash, and E.L. Connolly, 2006.
Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper. *Planta*, 223, 1178–1190.

Nikolic, M., S. Cesco, V. Römheld, Z. Varanini, and R. Pinton, 2007.
Short-term interactions between nitrate and iron nutrition in cucumber. *Functional Plant Biology*, 34, 402–408.

Oosterhuis, D., 2009.
Foliar fertilization: mechanisms and magnitude of nutrient uptake. Paper for the Fluid Fertilizer Foundation meeting in Scottsdale, Arizona. February 15-17, 2009.

Rellán-Alvarez, R., J. Abadía, and A. Alvarez-Fernández, 2008.
Formation of metal-nicotianamine complexes as affected by pH, ligand exchange with citrate and metal exchange. A study by electrospray ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 22, 1553–1562.

Römheld, V. and H. Marschner, 1986.
Evidence for a Specific Uptake System for Iron Phytosiderophores in Roots of Grasses. *Plant Physiology*, 80, 1175–1180.

Roschzttardtz, H., G. Conéjéro, F. Divol, C. Alcon, J-L Verdeil, C. Curie, and S. Mari, 2013.
New insights into Fe localization in plant tissues. *Frontiers in Plant Science*, 4, 1–11.

Roschzttardtz, H., L. Grillet, M.P. Isaure, G. Conejero, R. Ortega, C. Curie et al., 2011.
Plant cell nucleolus as a hot spot for iron. *Journal of Biological Chemistry*, 286, 27863–27866.

Sattelmacher, B., 2001.
The apoplast and its significance for plant mineral nutrition. *New Phytologist*, 149, 167–192.

Schonherr, J., 2006.
Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. *Journal of Experimental Botany*, 57, 2471–2491.

Susín, S., A. Abadía, J.A. González-Reyes, J.J. Lucena, and J. Abadía, 1996.
The pH requirement for in vivo activity of the iron-deficiency-induced “turbo” ferric chelate reductase: a comparison of the iron-deficiency-induced iron reductase activities of intact plants and isolated plasma membrane fractions in sugar beet. *Plant Physiology*, 110, 111–123.

Tarantino, D., P. Morandini, L. Ramirez, C. Soave, and I. Murgia, 2011.
Identification of an *Arabidopsis* mitoferrinlike carrier protein involved in Fe metabolism. *Plant Physiology and Biochemistry*, 49, 520–529.

von Wiren, N., S. Klair, S. Bansal, J.F. Briat, H. Khodr, T. Shioiri, R.A. Leigh, and R.C. Hider, 1999.
Nicotianamine chelates both Fe III and Fe II. Implications for metal transport in plants. *Plant Physiology*, 119, 1107–1114.

Werft van der P. and D. Dekkers. 1996.
Biological processes and phosphorous. Abstract E8, 11th IFOAM Scientific Conference, 11–15 Aug, Copenhagen, Denmark.

White, P.J. and M.R. Broadley, 2011.
Physiological limits to zinc bio-fortification of edible crops. *Frontiers in Plant Science*, 2, 80.

Wu, H., L. Li, J. Du, Y. Yuan, X. Cheng, and H-Q. Ling, 2005.
Molecular and biochemical characterization of the Fe(III) chelate reductase gene family in *Arabidopsis thaliana*. *Plant and Cell Physiology*, 46, 1505–1514.

Yeats, T.H. and J.K.C. Rose, 2013.
The formation and function of plant cuticles. *Plant Physiology*, 163, 5–20.

Yu, Q., C. Tang and J. Kuo, 2000.
A critical review on methods to measure apoplastic pH in plants. *Plant and Soil*, 219, 29–40.

Zekri, M. and G. England, 2010.
Foliar feeding and SAR for citrus trees. University of Florida, http://citrusagents.ifas.ufl.edu/archived_presentations/fall_prg2010/Citrus Agents Web Site-Nutritional.pdf.

More information: www.vfrc.org

Virtual Fertilizer Research Center
1331 H Street, NW
11th Floor
Washington, D.C. 20005
USA
E-mail: contact@vfrc.org

Indian Agricultural Research Institute
New Delhi