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Research Review Article

Phytosiderophore-based molecular approach for enhanced iron acquisition to increase crop production under high *p*H calcareous soils

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ABSTRACT

In the plant kingdom, two different Fe-deficiency chlorosis induced root strategies exist. Strategy I typical for dicots and monocots, with exception of grasses, is characterized by increased root reducing capacity (plasma membrane bound reductase), lowering the *p*H of the medium and in some instances enhanced release of phenolics. Strategy II iron acquisition mechanism of graminaceous monocots release iron-chelating mugineic acid family phytosiderophores (MAs in response to Fe deficiency) which solubilize inorganic Fe III compounds by chelation to form Fe^{3+}-MA. These Fe^{3+}-MAs are passed through a highly specific Fe transporter (yellow strip – YS I) present in the root plasma membrane. Further, notification of the key enzymes such as nicotianamine synthase (NAS) and/or nicotianaimine aminotransferase (NAAT) as well as deoxygenase gene IDS3 paved the way for the development of transgenic rice plants with enriched DMA both in shoot and root of chlorotic plants. Therefore, it is established that the genetic engineering can transfer traits from plants tolerant to adverse conditions to field crops for sustained productivity. Introduction of NAS, NAAT and IDS3 genes, respectively in rice has been found very effective in producing higher amount of phytosiderophores (MAs). Introduction of linearly combined genes NAS, NAAT and IDS3.Two cis-elements (iron deficiency-responsive elements) IDE 1 and IDE 2 have also been identified.

Key words: Calcareous soil, Iron acquisition, Molecular approach, Phytosiderophore

Iron being an important micronutrient acts as a limiting factor in crop production even when plants do not show visual deficiency symptoms. Therefore, green plants must have continuous iron supply during the early stage of crop growth and development of new leaves and shoots because iron is not transferred from older to younger leaves. The law of maximum plant yield (Wallace 2000) states that limiting factors interact in a sequentially additive manner; so that more response is obtained by the use of each unit.

There appears to be some more alternatives to strengthen strategy II mechanism of iron to:

(i) device the technology to manufacture polyolefin-coated resin controlled release Fe fertilizer (Yahuda *et al.* 2003).

(ii) develop synthetic MA analogue for commercial use (Mori *et al.* 2000).

(iii) produce transgenic plants with increased efficiency of phytosiderophore secretion (Mori *et al.* 2003).

IRON ACQUISITION MECHANISMS

Lime induced chlorosis is a major agricultural problem leading to reduced crop yield in calcareous soils which cover about 30% of the world cultivated area (Chen and Barak 1982). Most of iron in such soils forms sparingly soluble Fe^{3+}- precipitates mainly as derivatives of $Fe(OH)_3$ due to high *p*H conditions (Inoue *et al.* 1993) lowering the solubility (Brady and Weil 1996) and availability of Fe to the plants (Oki *et al.* 1999). Based on Fe acquisition, higher plants have been classified into two categories: strategy I and strategy II (Marschner *et al.* 1986).

Strategy I of non- graminaceous plants

It is an iron acquisition mechanism present in higher plants except graminaceous monocots. Non-graminaceous plants under Fe deficiency conditions (*i*) increase their ferric reductase activity at the root surface, (*ii*) enhance proton (H^+) excretion to lower down the *p*H, and (*iii*) release of reductants or chelators (citrate, malate, phenolic and other organic acids)

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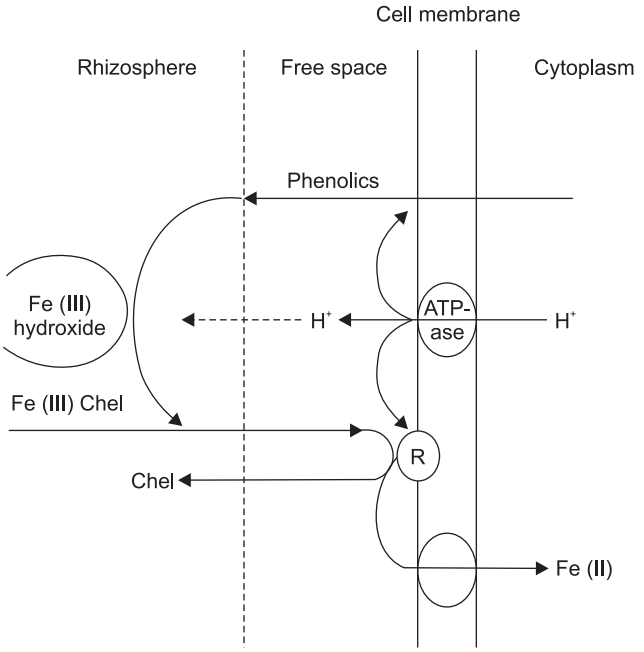


Fig 1 Fe acquisition mechanism of strategy I (Romheld and Marschner 1986) modified by Mori (1998)

in the rhizosphere consequently elevating iron availability in the rhizosphere to enhance iron acquisition (Fig 1).

Strategy II of graminaceous plants

It is associated with the roots of graminaceous plants (Fig 2). The exudation of phytosiderophores by strategy II plants is known to increase Fe solubility due to their relatively high stabilities. The phytosiderophores also exhibit high affinity for Zn, Mn and Cu (Table 1) (Wallace 2000).

Iron-deficient graminaceous plants secrete MAs into the rhizosphere, and these natural chelators bind with sparingly soluble Fe (III) in the soil medium converting it to water soluble Fe^{3+}-MAs; then transporting Fe into the root cells through a highly specific Fe (III) transporter [(yellow strip-YS1 (Chen 1997)] for Fe^{3+}-MAs (Mihashi *et al.* 1991).

Discovery of mugineic acids

The knowledge of strategy II iron acquisition mechanism has increased considerably with the discovery of mugineic acid (Takagi 1976). MAs consist of compounds (Fig 3) mugineic acid (MA), avenic acid (AVA), distichonic acid (DA), 2-deoxymugeneic acid (DMA), 3-hydroxymugineic acid (HMA), 3-epi hyroxydeoxymugeneic acid (epi-HDMA)

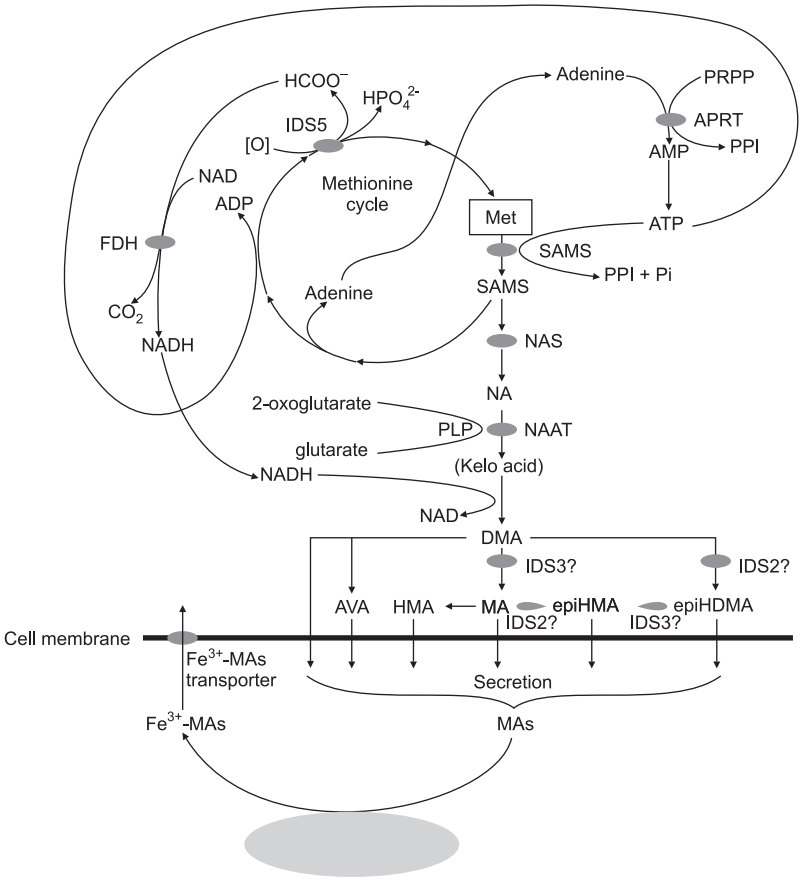


Fig 2 Fe acquisition mechanism of strategy II plants (Romheld and Marschner 1986) modified by Mori (1998)

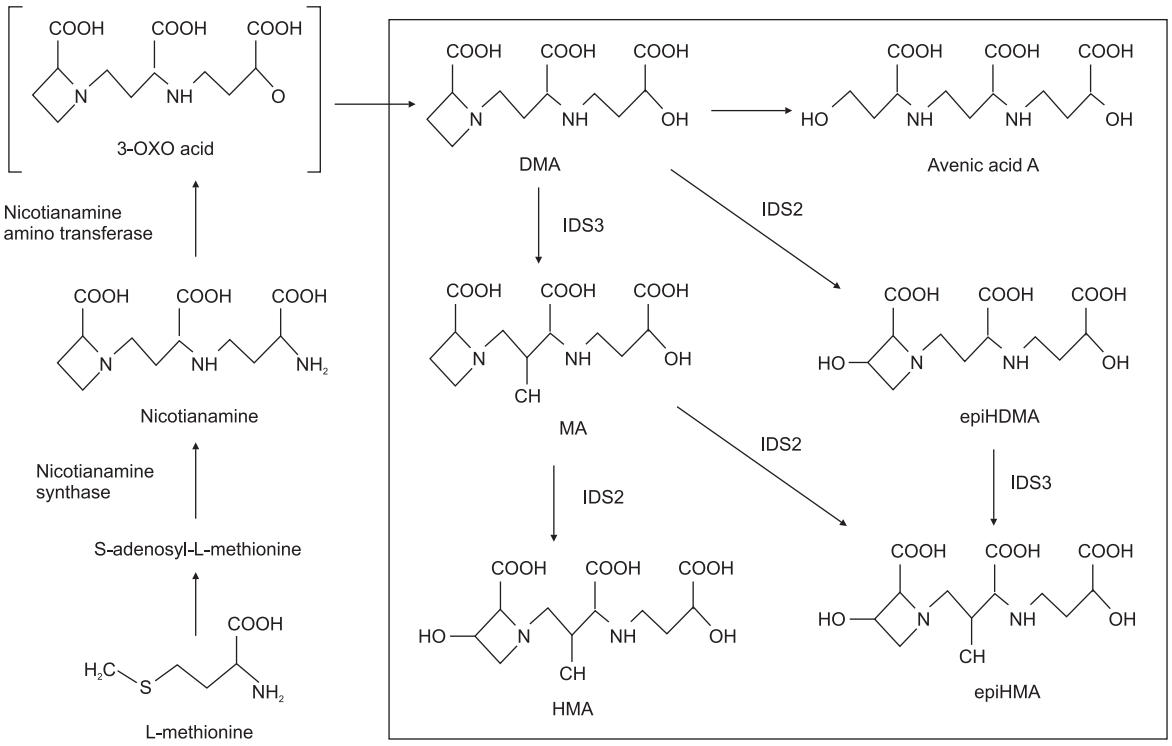


Fig 3 Hypothetical metabolic pathway for the synthesis of mugeinic acid family phytosiderophores (Nakanishi *et al.* 2000).

(Ma *et al.* 1999). Recently identified MAs, 3-hydroxy-2-deoxymugenic acid (HDMA) seems to be an epimer of epi-HDMA (Ueno *et al.* 2004).

Biosynthetic pathway of mugineic acid phytosiderophores

Biosynthetic pathway from methionine to MAs (Higuchi *et al.* 1996) has been established (Fig 3) and methionine has been recognized as precursor (Mori and Nishizawa 1989). Subsequently chemical intermediates in the steps from methionine to MAs have also been identified. S-adenosylmethionine (SAM) is synthesized from methionine by SAM synthetase. Then nicotianamine synthase (NAS) combines three molecules of SAM to form one molecule of nicotianamine (NA) (Higuchi *et al.* 1996). NA is then converted to 3-keto acid by nicotianamine aminotransfease (NAAT). Finally, deoxymugeneic acid is synthesized by an unknown reductase. Other MAs are formed by hydroxylation of DMA (Nakanishi *et al.* 2000). Further iron deficiency specific clone-3 (IDS3) enzyme hydroxylates the C-2 position of DMA and epi- HDMA, whereas IDS2 hydroxylates the C-3 position of MA and DMA.

Methionine source in biosynthetic pathway: Mehionine is supplied to roots through methionine cycle (Yang cycle) (Ma *et al.* 1999). The cycle generates methionine as precursor for ethylene synthesis in the event of environmental stress (Yang and Hoffman 1984). Though, methionine has been detected in the phloem of rice and wheat leaves (Hayashi and Chino 1986, 1990) but it is not translocated to roots and is supplied in the root itself (Nakanishi *et al.* 1999).

Gene cloning of MAs synthesis: Most of the genes involved in MA synthesis have been cloned. Among them S-adenosylmethionine synthetase (HvSAMS) (Daufault, 1987), nicotiamine synthase (HvNAS) (Higuchi *et al.* 1999a) and nicotianamine aminotransperase (HvNAAT) (Takahashi *et al.* 1999) gene encode enzyme for the biosynthesis of 2'-doxymugeneic acid, the first member of MAs in biosynthetic pathway. Two genes, iron deficiency specific clone no. 2 (IDS2) (Okumura *et al.* 1994) and IDS3 (Nakanishi *et al.* 1999) encode deoxygenases which hydroxylate C-3 and C-2 positions of MAs, respectively (Kobayashi *et al.* 2001). Some genes involved in recycling of methionine have also been cloned as IDH (Yamaguchi *et al.* 2000a), the formate dehydrogenase gene HvFDH (Suzuki *et al.* 1998) and the adenine phosphoribosyl transferase (Hv APRT) (Itai *et al.* 2000). The expressions of these genes are induced in Fe deficiency in roots (Negishi *et al.* 2002). Besides, Fe deficiency inducible genes IDS1 (Okumura *et al.* 1991), IDS2 (Yamaguchi *et al.* 2000b) and the tonoplast located ABC transporter IDI 7 (Yamaguchi *et al.* 2002) have also been cloned. In addition to these two novel cis-elements, IDE1 and IDE2 have also been identified as a potential tool to know the molecular mechanism regulating Fe homeostasis in higher plants (Kobayashi *et al.* 2003).

Fe (III)-transporter: A hypothetical "Fe^{3+}-MA transporter" was based on the findings (*i*) translocation of ^{59}Fe to the top of the rice plant supplied with $^{59}Fe^{3+}$-MAs was much faster

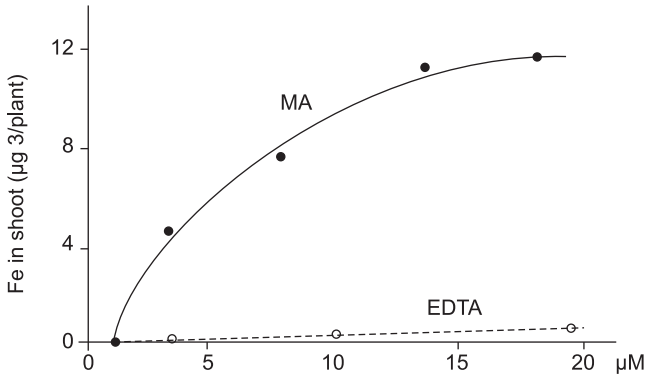


Fig 4 Effect of mugineic acid (MA, solid line) or EDTA (broken line) on the absorption of iron by young rice roots (*Oryza sativa* cv. Norin 16, *p*H 7.0). Data were obtained 24 hr after $^{59}FeCl_3$ supply (Takagi *et al.* 1984).

than that of $^{59}Fe^{3+}$-EDTA (Fig 4), (*ii*) the rate of absorption of $^{59}Fe^{3+}$-MA by the Fe deficient barley plant roots was markedly higher than that of Fe sufficient barley roots (Fig 5), and (*iii*) BPDS did not inhibit the absorption of $^{59}Fe^{3+}$-MA (Romheld and Marschner 1986). Four proteins labelled with ^{35}S-methionine within the membrane fraction of maize, wild-type Alice, mutant YS 1 were identified (von Wiren *et al.* 1997), which had a defect in Fe^{3+}-MA transporter activity. Recently a gene encoding Fe^{3+}-MA transporter yellow stripe (YS 1) was cloned in maize (Curie *et al.* 2001). A positive YS 1 like gene Os YS 1, 2 identified (Nishizawa 2004) was not a rice Fe^{3+}-DMA transporter for iron uptake from the soil but a transporter involved in the long distance transport of iron. Similarly, Fe^{2+} transfer gene Os IRT 1 (Ishimaru *et al.* 2004) was found to be involved in Fe^{2+} distribution and Fe^{3+} uptake from the soil.

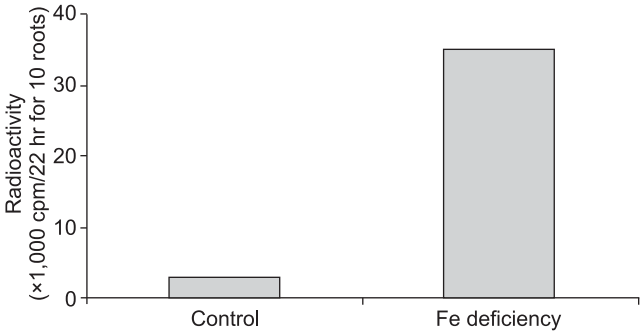


Fig 5 Iron uptake activity measured by the multicompartment root box method

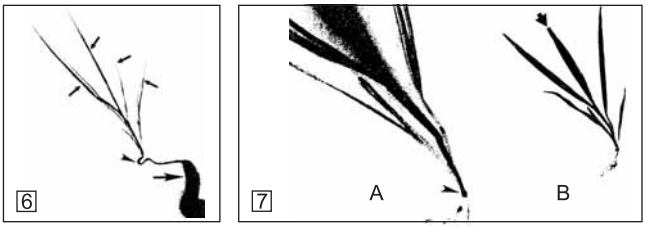
FACTORS AFFECTING STRATEGY II

Iron transport in the roots of strategy II plants

Fe-deficient graminaceous plants secrete MAs into the rhizosphere, and this natural chelator binds with sparingly soluble Fe (III) in the soil medium converting it to water-soluble Fe (III)-MAs; then transporting Fe into the root cells through a highly specific transport system Fe (Fig 5). The specific transporter was proposed based on the indirect evidence that root membrane transport of ^{59}Fe supplied as ^{59}Fe (III)-MAs was not inhibited by the strong synthetic Fe (II)-chelator, basophe-nanthroline disulfonate (BPDS).

Iron transport from root to shoot

The iron-uptake rate from roots to shoots is increased more than 10-fold when Fe is supplied by Fe (III)-MAs compared with Fe (III)-EDTA or Fe (III)-desferal in rice or barley. Radioactivity in the new leaves of barley was only within 10 min. after supplying ^{59}Fe (III)-epiHMA in the water-culture solution. After 4 hr treatment, Fe was strongly transported to the tiller, the newest leaf, and other leaves. Thus, tiller(s) and newly developing leaf are recognized as strong sinks for Fe. Iron accumulation was dependent on the age of the leaves. Iron absorbed from one root is also translocated to the other roots. When Fe was absorbed from a single new root, Fe was transported to the other roots, especially to the root tips. The sectioning and staining or scanning electron microscopy image of the Fe-deficient root tips revealed the emergence of many root primordia. Therefore, root meristems are doubtless a strong sink of Fe. When Fe was absorbed from an old single root, Fe was transported evenly to the other roots. Absorbed Fe from a single root was also transferred to the corresponding leaves, but first only to the veins (thin arrows) directly connected to that root. The labeled vein is not always the central vein but sometimes a peripheral vein. An interesting phenomenon was observed; *i e* Fe from the roots deposited at once to the end of the basal part of the leaves where meristematic cells exist. This meristematic zone will develop to leaves or roots in the future. Therefore, this part also seems to be the place where the distribution of Fe is controlled; to which Fe coming either from a leaf or from a root be distributed. Therefore, this zone is called the 'discrimination centre' of nutrient transport (Figs 6, 7).



Figs 6–7 **6.** Transport of Fe from an old single root (big arrow) to other roots and leaves. Special veins of the special leaves (small arrows) are labelled although other roots are evenly labeled. Arrow head shows a discrimination centre. **7.** Transport of Fe of 4 hr supply from Fe sufficient barley leaf. The discriminatin centre (arrow head) is stongly labelled. Leaf veins are asymmetrically labelled. (*A*) Radio autograph (*B*) gross image of the plant.

Iron transport from shoot to root

When Fe was supplied from the cut part of a leaf of the main shoot, Fe was transported through the phloem to the

basal part of the leaf and retranslocated to the roots and other leaves, especially the tillers. Most of the absorbed Fe was mainly transported through an apoplasmic mechanism and partly through the veins. Within 4 hr, the front of the transported Fe is still in the middle part of the leaf, and after 8 hr, it reaches the basal part of the leaf. Retranslocation of Fe throughout the discrimination centre is first to the tillers and then to the newest leaf of the main shoot. Transport of Fe to the root meristems is very clear in case of Fe-deficient plants. This specific Fe transport from shoot to root is very similar to the phosphate transport from shoot to root.

Effect of iron status on iron transport in plants

The role of Fe (III)-MAs in Fe transport into plant roots has been studied in detail. However, no information is available on their role in Fe transport in the foliage of the plants despite the presence of MAs in plant shoots. Moreover, the environmental factors that regulate Fe transport in higher plants have not been studied.

Distribution of ^{49}Fe in the shoots between two rice plants, one cultured with and without iron was compared by supplying "Fe (III)-epi HMA in the culture medium. After absorption for 4hr, radioactivity in the Fe-deficient rice shoot was stronger than that in the Fe-sufficient one. After 12 hr, the difference could still be observed. However, after 24 hr. the difference was no longer clear. These data coincide with the fact that Fe-deficient graminaceous plants can absorb and transport much higher amount of Fe than Fe-sufficient plants. This is probably caused by the increased activity of the (Fe (III)-epiHMA)-transporter in the Fe-deficient root cells and the increase in shoot demand for Fe as well.

Effect of iron oxides on solubility of Fe in calcareous soils

Iron solubility in soils is affected by many factors but the presence of secondary Fe (III) oxides is of great importance. The yellow and red colours usually be traced the presence of oxide coatings over soil particles. Primary minerals containing Fe(II) are generally unstable in soils and slowly weather in the presence of atmospheric oxygen. Released Fe^{2+} to Fe^{3+} precipitates as Fe(III) oxides and hydroxides arranged in the order of descending solubility: $Fe(OH)_3$ amorphous > $(OH)_3$-soil Fe> Fe_2O_3 (Maghemite)> $Fe(OH)_3$ (Lepidocrocite) >Fe_2O_3 (Hematite) >$Fe(OH)_3$ (Goethite) having log KO values ranging from 3.5 (the most soluble amorphous $Fe(OH)_3$) to 0.02 (the least soluble $Fe(OH)_3$ oxide). This extremely low solubility explained the difficulty of keeping Fe^{3+} soluble and mobile (Lindsay 1995).

In fact, initially Fe^{3+} precipitates as an amorphous and high surface $Fe(OH)_3$ products, which gradually transforms to a more orderly and less soluble product corresponding to soil -Fe. Among the Fe (III) solid phases ferrihydrite contributed to the solubility reflected by soil-Fe, whereas increasing *p*H and redox precipitated it initially as amorphous Fe $(OH)_3$, which slowly changed to soil-Fe. Only under much stabilized *p*H and oxidizing conditions soil-Fe disappeared and more insoluble crystalline iron oxides remained to control Fe^{3+} solubility.

Fortunately Fe^{3+} reacted in aqueous system to form very stable hydrolysed species raising total soil Fe^{3+}. The minimum Fe solubility occurred in the *p*H range of 7.5-8.5 where major solution species was $Fe(OH)_3$ and Fe deficiency can be expected in all aerated soils. Calcareous soils are strongly buffered in the *p*H range of about 8.0 by bicarbonates where Fe had its minimum solubility. Hence, chlorosis is appropriately referred to as lime induced chlorosis.

MAs secretion, adsorption and absorption of chelated MA-Fe

Calcareous soils varied with respect to their Fe extraction efficiency (Table 1) and soils having higher extraction efficiency might be having amorphous form of Fe (Singh *et al.* 1992), whereas soils showing poor extraction efficiency might be having crystalline form of Fe (Schwertman 1991). High MA adsorption on iron oxides at low *p*H and adsorption value decreased with increasing *p*H showing negligble value at *p*H 10 (Inoue *et al.* 1993). In fact, MA adsorption on Fe oxides was related to specific surface area and crystalinity (Inoue *et al.* 1993).

Table 1 Effect of mugineic acid and other chelators on solubility of Fe, Zn, Mn and Cu (n mol/g soil) solubilized from soil shaken 1 hr with 20 mL deionized water containing 1 n mol chelators in Indian calcareous soils

Chelator	Solubility (n mol/g soil)			
	Fe	Zn	Mn	Cu
DTPA	36.3	12.0	14.2	–
FOB	22.2	–	10.4	–
MA	52.6	11.2	7.7	3.6

MA adsorption on Fe iron oxides increased in the order : ferrihydrite > goethite > lepidocrite > hematite between *p*H 3-7. However, at *p*H >10, MA-Fe complexes were decomposed to MAs and $Fe(OH)_3$ colloids. The dissolution pattern thus followed the descending order of ferrihydrite > hemidocrocite > hematite-goethite. The maximum amount of Fe dissolved by MA was in the *p*H range of 7-8 (Fig 8). These dissolved MA-Fe complexes by MA from Fe oxides may be absorbed by graminaceous plants in calcareous soils.

Chemical structures, biosynthetic pathway of mugineic acid (ma)-related phytosiderophores and crop cultivars

The identification of Met as a precursor of MA raised the every possibility of producing transgenic cultivars by inducing the genes of MAs biosynthesis into cultivars susceptible to Fe deficiency. Interestingly, among the graminaceous plants, the degree of tolerance to Fe deficiency positively correlated with the capacity to produce and secrete

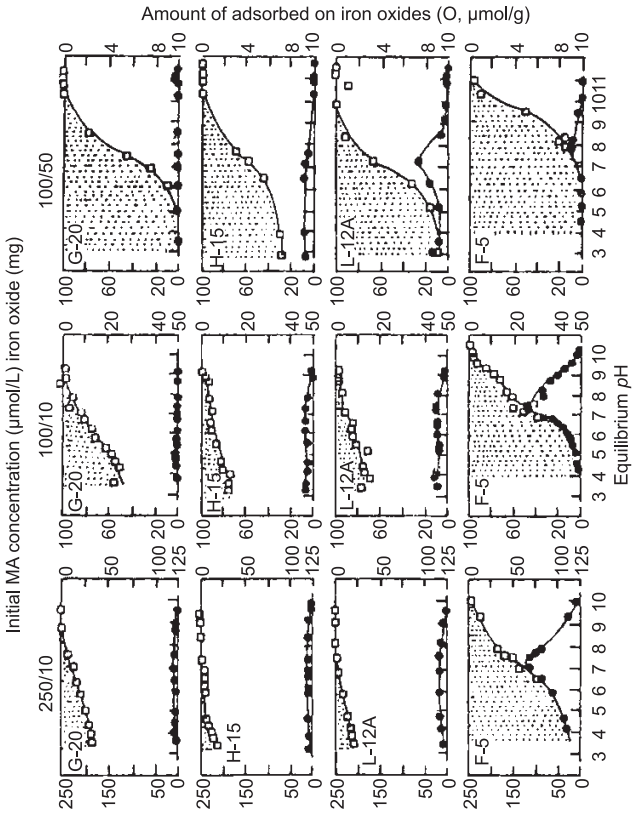


Fig 8 Adsorption of mugineic acid (MA) by Fe oxides and the concentrations of total MA in solution and Fe dissolved from Fe oxides as influenced by *p*H. The doted area indicated the amount of MA adsorbed on each Fe oxide: G-20= Goethite; H-15= hematite; L-12A= tepidocrocite; F-5= terrihydrite. (Inoue *et al.* 1993).

MAs into rhizosphere under iron-deficient condition in the order, barley > rye > wheat > oat > maize > sorghum>rice (Singh *et al.* 1992) though crop cultivars also varied (Table 2) depending upon root morphology and quality of MAs produced (Singh *et al*. 1992). Virtually, this research opened the door for the introduction of one of the key enzymatic genes such as nicotianamine synthase (NAS) or nicotianamine aminotransferase (NAAT) or other subsequent hydroxylation enzymes for MAs synthesis from DMA (Nakanishi *et al*. 1997) which played a key role in developing transgenic plants, specially monocotyledons tolerant to Fe deficiency (Mori 1996).

Although dicotyledons (dicots) also possessed NAS activity (Higuchi *et al.* 1999a) but they apparently lacked NAAT activity as well as transporter protein in the plasma membrane of root cells. Therefore, by introducing NAAT gene from cereals to dicots, it would be possible to develop dicots with ability to produce DMA. These plants will favourably increase not only soluble Fe in the rhizosphere in the form of Fe^{3+}-DMA to DMA secretions from roots of the transgenic plants but at the same time may also solubilize other micronutrients, viz. Cu, Zn, Mn too. Further, study of

Table 2 Qualitative and quantitative analysis of mugineic acid family phytosiderophores (MAs) from Indian gramineae plants

Crop cultivar	Days after planting	Secreted MAs (µg/plant^6/hr^3)				
		Epi-HMA	HMA	MA	DMA	A
Maize	28				51	1
Deccan 103	35					
	40					
Barley	30			1 190	297	1
NBD 209	35			1 640	678	
	40			1 860	602	21
Oat	31					306
PO 3	35	3			12	186
	45					815
Russian	36	154	257	110	641	
Rye	39	255	285	143	1 160	
	42	291	503	90	109	56
Sorghum	39	2	8		12	2
PKSS 6	42	3	2		3	1
	45	>1	13	1	13	2
Wheat	32				2 830	
KRL 1–4	39			20	1 730	1
	42				3 350	
Wheat	32				2 796	
HD 2329	35				2 349	
	40				2 420	

NAAT activity has demonstrated the existence of two isoforms of NAAT with different physiological significance. NAAT-I provided the required activity in the emergency (Fe starvation) and therefore, this is important gene for Fe deficiency response mechanism and can be used as an ideal modeling tool for molecular breeding. NAAT-II works at the basal level of MAs production (Kanazawa *et al.* 1994).

Effect of light and temperature on MAs secretion and its energy dependence

Fe-deficient experimental evidences suggest that the initiation and regulation of' MA release is not only due to light signal but rather more inclined to synchronous temperature increase with illumination. The most dominant factor that acts as trigger for the initiation of MAs release is the time of temperature increase. Therefore, in general, wider variation in temperature leads to sharper peak of MAs release (Takagi 1991). It has been observed that under continuous light well defined MAs release peak is obtained (Fig 9a) whereas under constant temperature poorly defined MAs release peak is observed (Fig 9b). Further, under standard light cycles with reverse temperature condition, MAs release peak shifts from morning to evening hours (Fig 9c). It has also been noticed that MAs secretion is ATP dependent as the release is inhibited by ATPase inhibitor DCCP (dicyclohexycarbodiamide).

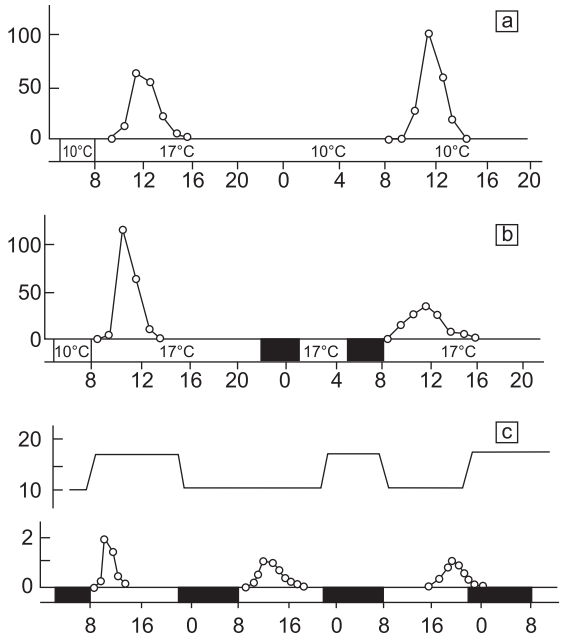


Fig 9 Secretion pattern of mugeneic acid from Fe-deficient barley roots under three different culture conditions; (*a*) Continuous light and usual temperature change, (*b*) 14 hr light under constant temperature, (*c*) 14 hr light 10 hr dark under reversed temperature conditions. (Takagi 1991)

FE^{3+} MOBILISATION BY CELL MEMBRANE PHOSPHOLIPIDS

It has been observed that MAs release and F^{3+} MA transport was high enough not only to overcome to meet the demand of competiting Fe chelation by microbial siderophores and degradation by microbes but also inhibits the Fe^{3+}-MA binding to the phospholipids of the plant root cell plasma membrane (Mihashi *et al.* 1991).

EFFECT OF Fe SHOOT AND ROOT CONCENTRATION OF CHLOROTIC PLANTS ON MASS SECRETION

In general, chlorotic leaves have high Fe concentration and it may be partly because of concentration effect due to strong inhibition of shoot growth under deficiency conditions. It may also be partly due to inactivation of Fe in the leaves viz. Fe^{3+} to Fe^{2+} reduction inhibition (Olsen and Brown, 1981). Higher phosphorus translocation to shoot in Fe deficient plant may lead to the precipitation of Fe-P in the system (Cumbus *et al.* 1977). Any plant with leaf concent-ration below 40-70 mg Fe/kg dry weight could respond to Fe favourably (Wallace 1995). However, total concentration in the shoot is not an adequate parameter for the physiological availability of Fe as leaf chlorophyll contents are negatively correlated to N, P, K, Fe (Koseoglu 1995) and therefore cannot be taken as reliable index for assessing Fe chlorosis. However, extractable Fe, Fe index, P/Fe and K/Ca are reliable criteria for assessing Fe chlorosis. High shoot and root Fe concentration in tomato plants showing visual deficiency as plants were unable to synthesize nicotianamine required for Fe utilization (Pich *et al.* 1991).

The chlorotic plant roots also have high Fe concentration and this small magnitude of enhancing effect of Fe root concentration may be due to microbial chelation, mobilisation and Fe transport to roots (Bar-Ness *et al.* 1992, Drechsel *et al.* 1991, Shenker *et al.* 1992). Obviously the chelator is decomposed by microbes at root surface and released Fe is adsorbed to cell wall adsorption sites or precipitated as Fe $(OH)_3$.

EFFECT OF Zn DEFICIENCY ON MASS SECRETION

Intact Zn-deficient wheat plants enhanced phytosiderophore release due to impaired translocation of Fe to the shoot. Zn deficiency might have affected xylem loading or xylem Fe transport resulting into less Fe transport from the root to the shoot as roots suffer from physiological Fe deficiency. Induction of Fe deficiency due to excessive Zn supply in young corn leaves is known possibly due to replacement of Fe by Zn^{2+} in Fe-dependent process without affecting total Fe concentration (Rosen *et al.* 1977). Zn-deficient plants may impair protein synthesis as high levels of amino acids might have sequestered Fe in unavailable form (Kitagishi and Obata 1986). Further, higher translocation of P to shoot in Zn-deficient plants (Marschner and Cakmak 1986) might have led to Fe-P precipitation in the xylem (Cumbus *et al.* 1997). The role of Si, in cation-anion balance may also be involved in regulation of Fe in gramineae plants (Marschner *et al.* 1986).

MICROBIAL DECOMPOSITION OF MA

Contribution of microbial siderophores to Fe acquisition and translocation by gramineae plants depends on cultural conditions (Crowley *et al.* 1992). However, the main compounds which solubilizes Fe in the rhizosphere of grasses are the MAs. In general, microbial siderophores have higher affinity with Fe^{3+} than phytosiderophore. Therefore, it is possible that the increasing microbial population may result in higher siderophore production leading to microbial degradation of phytosiderophore. Pseudomonas was identified as dominant bacteria responsible for higher degree of decomposition using MAs as a sole carbon source. The decomposed MAs followed the descending pattern of >DMA>MA>epi HMA and the same order was noticed in calcareous soils. This is actually one of the reasons for poor MAs secretion in maize and sorghum which secrete DMA liable for higher degree of degradation. It was also found that maize and sorghum being C_4 plants have certain diazotrophic bacteria *(Azospirillum strains)* which preferably live in the rhizosphere/ rhizoplane of C_4 species utilizing phytosi-deroshores as a source of Fe.

The bacteria also decomposed Fe^{3+}-MA. However, plants

Table 3 Effect of different treatments on iron uptake by rice

Treatment	Iron accumulation (mg/hill)					
	Grain		Straw		Total	
	2001	2002	2001	2002	2001	2002
Application method						
Uniform	0.79	0.80	0.95	1.00	785	8.21
Co-situs	0.83	0.84	0.99	1.05	8.04	8.50
SEm ±	0.01	0.01	0.02	0.02	0.12	0.11
CD (*P*=0.05)	NS	NS	NS	NS	NS	NS
Fertilizer source						
NPK + 100% pyrite	0.69	0.70	0.81	0.84	7.02	7.31
NPK + 100% PRCSR Fe*	0.85	0.86	0.95	1.01	7.77	8.22
NPK + 50% pyrite + 50% PRCSR Fe*	0.87	0.88	1.07	1.12	8.41	8.80
NPK + 75% pyrite + 25% PRCSR Fe*	0.87	0.88	1.10	1.16	8.79	9.28
NPK + 25% pyrite + 75% PRCSR Fe*	0.77	0.77	0.93	0.99	7.72	8.15
SEm+	0.02	0.02	0.03	0.02	0.19	0.18
CD (*P*=0.05)	0.06	0.05	0.08	0.07	0.56	0.52
Control	0.65	0.66	0.74	0.79	6.10	6.45
SEm ±control vs. others	0.03	0.03	0.04	0.04	0.28	0.26
CD (*P*=0.05)	0.09	0.08	0.12	0.11	0.83	0.77

avoid decomposition of MAs or Fe^{3+}-MA from bacteria as microbial activity in the rhizosphere and at the rhizoplane usually increases from apical to basal root zone, whereas MAs release and Fe^{3+}-MA absorption is mainly confined to apical root zones (Romheld 1991).

DEVELOPMENT OF TRANSGENIC PLANTS

Fe-deficient the strategy I and the strategy II mechanisms are correlated to efficient crop cultivars (Singh *et al.* 1992a), which need rigorous and continuous screening to develop efficient plants (Fig 10) to produce higher amount of qualitative phytosiderophores with exceptionally low level of chlorosis. Nevertheless, it is laborious, time-consuming and site specific. Besides, there are other ways namely soil amendments (gypsum), crop management, fertilizer application, synthetic chelators and microbial chelators to meet the insufficient Fe supply for the plants. Though chelators are the efficient source of iron (Chen 1997), these are leachable and unstable due to negative charge and therefore, repeated applications are required making the application cost-effective. Besides, there is risk prone to contaminate groundwater (Yahuda *et al.* 2003).

Polyolefin-coated resin controlled release fertilizer has a good future (Goos *et al.* 2004; Singh *et al.* 2006) because the release of nutrient from resin coating is controlled only by moisture permeability of resin coating. Therefore, these fertilizers may have greater stability and solubility due to highly controlled release of nutrient in harmony with the need of the crop (Nanzyo *et al.* 1997, Yahuda *et al.* 2003) enriched quality produce (Welch 2000) even with reduced rate of application (Table 3, Gauertal 2000) because of their closer multiple contacts with roots and particles of fertilizer

Fig 10 Newly developed non-transgenic (control) and transgenic (NNAT) rice plant.

materials (Nanzyo *et al.* 1997) as envisaged by experimental results (Singh *et al.* 2006, Singh *et al.* 2007, Table 3).

FUTURE RESEARCH PLAN

Production of transgenic plant with strong Fe^{III}-MA transporter gene will be an ideal approach because Fe^{II}-MAs are formed at the root surface just after MAs secretion and then immediately absorbed and transported simultaneously through Fe^{II}-MAs transporter. Therefore, all secreted MAs

will be taken up without much loss. Thus, MAs transporter gene will be sufficient to compensate even the low amount of secreted MAs in crops like rice, sorghum.

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