Review

Recent insights into iron homeostasis and their application in graminaceous crops

By Takanori KOBAYASHI,[*1] Hiromi NAKANISHI[*1] and Naoko K. NISHIZAWA[*1,*2,†]

(Communicated by Teruhiko BEPPU, M.J.A.)

Abstract: Higher plants utilize various mechanisms to maintain iron homeostasis. To acquire sparingly soluble iron from the rhizosphere, graminaceous plants synthesize natural iron (III) chelators known as mugineic acid family phytosiderophores (MAs). Recent research has uncovered various genes involved in iron uptake and translocation, as well as factors regulating the expression of these genes, especially in rice. Manipulation of these molecular components is used to produce transgenic crops with enhanced tolerance to iron deficiency, or with a high seed iron content. Since iron homeostasis is closely linked to that of other mineral elements, an understanding of this phenomenon will serve as the basis for the production of crops with low concentrations of toxic metals and transgenic plants for phytoremediation.

Keywords: gene regulation, iron deficiency, mugineic acid family phytosiderophores, rice, graminaceous plants, chelators

1. Introduction

Iron is essential for most living organisms, including plants. Despite its abundance in the Earth's crust, iron is sparingly soluble under aerobic conditions, especially in high pH and calcareous soils, which account for about 30% of the world's cultivated soils. Thus, iron deficiency is a widespread agricultural problem that hinders plant growth and lowers crop yields.[1),2)] Because plants are the primary food source for humans, the nutritional state of plants is of central importance to human health.[3)] To acquire enough iron while avoiding toxicity, plants tightly control their uptake, utilization, and storage of iron in response to its availability in the environment.

Since the discovery of the iron solubilizing capacity of root washings from iron-deficient rice and oat plants by Takagi,[4)] the mugineic acid family phytosiderophores (MAs) have been identified as natural iron (III) [Fe(III)] chelators synthesized in the roots of graminaceous plants. Römheld and Marschner[5)] named this iron-acquisition mechanism, which is specific to graminaceous plants, 'Strategy II', in comparison with Fe^{2+} transport system after reduction of Fe(III) or 'Strategy I' in non-graminaceous plants. The genes involved in the MAs-dependent system have been identified over the past two decades. More recently, iron translocation within plants at the molecular level has begun to be understood. Also, regulatory components of the genes involved in iron uptake and translocation are being identified. In this review, we introduce recent discoveries related to these molecular components in graminaceous plants, and their application to produce transgenic plants with increased tolerance to iron deficiency, improved food quality and those applicable for remediation of heavy metal-polluted soils. Research on these subjects for rice has recently

[*1] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.
[*2] Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Ishikawa, Japan.
[†] Correspondence should be addressed: N. K. Nishizawa, Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi-machi, Ishikawa 921-8836, Japan (e-mail: annaoko@mail.ecc.u-tokyo.ac.jp).

Non-standard abbreviation list: bHLH, basic helix-loop-helix; DMA, 2′-deoximugineic acid; DMAS, deoxymugineic acid synthase; GUS, β-glucuronidase; IDE, iron deficiency-responsive element; IDEF, iron deficiency-responsive element-binding factor; MAs, mugineic acid family phytosiderophores; NA, nicotianamine; NAAT, nicotianamine aminotransferase; NAS, nicotianamine synthase; PETIS, positron-emitting tracer imaging system; RNAi, RNA interference; ZIP, zinc-regulated transporter, iron-regulated transporter-like protein.

doi: 10.2183/pjab.86.900

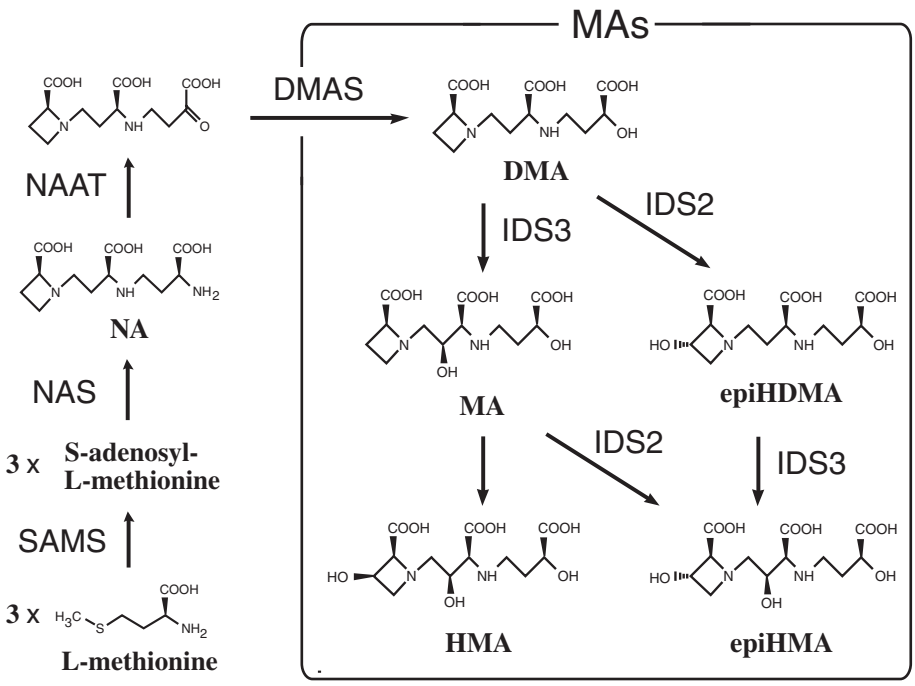

Fig. 1. The biosynthetic pathway of MAs in graminaceous plants. SAMS, S-adenosylmethionine synthetase; NAS, nicotianamine synthase; NAAT, nicotianamine aminotransferase; DMAS, deoxymugineic acid synthase; IDS2, iron-deficiency-specific clone no. 2; IDS3, iron-deficiency-specific clone no. 3; NA, nicotianamine; DMA, 2′-deoxymugineic acid; MA, mugineic acid; HMA, 3-hydroxymugineic acid; epiHDMA, 3-epihydroxy-2′-deoxymugineic acid; epiHMA, 3-epihydroxymugineic acid. To date, four other MAs have been identified.

made great advance due to the completion of the rice genome sequencing project and outstanding importance of rice as a staple food crop. Recent advances in iron nutrition in non-graminaceous plants have been reviewed elsewhere.[6)–8)]

2. Genes involved in iron uptake

The synthesis and secretion of MAs are specific to graminaceous plants and are strongly enhanced under low iron conditions. The identification of molecular components involved in the biosynthesis of MAs has been examined on the basis of extensive physiological studies focused on the identification of MAs and their biosynthetic pathways (reviewed by Refs. 2, 9, 10). To date, nine types of MAs have been identified, and their biosynthetic pathways beginning with methionine as well as the corresponding genes that encode each enzyme in the biosynthetic step have been largely established (Fig. 1).[2),9)–11)] Four sequential enzymatic reactions convert three molecules of L-methionine to 2′-deoximugineic acid (DMA). All graminaceous species examined thus far possess the ability to synthesize DMA, which is further hydroxylated to form other MAs in some species, including barley and rye. The methionine cycle works vigorously to meet the increased demand for methionine in the synthesis of MAs.

Most of the biosynthetic genes have been cloned first from barley, and then from rice, maize and wheat.[9),10)] The spatial expression patterns of the DMA biosynthetic genes in rice have been investigated by histochemical observation of promoter-β-glucuronidase (*GUS*) transgenic rice lines. Rice nicotianamine synthase genes *OsNAS1*, *OsNAS2*, nicotianamine aminotransferase gene *OsNAAT1*, and deoxymugineic acid synthase gene *OsDMAS1* show similar expression patterns in roots and leaves, with expression observed mainly in the phloem tissues of roots and leaves in the presence of adequate iron and strong induction throughout all root and leaf tissues in response to iron deficiency.[12)–14)] These results strongly suggest that DMA is synthesized in all root cells under iron-deficient conditions. A rice knockout mutant of *OsNAAT1* exhibited severe growth retardation under aerobic conditions and a complete defect in DMA secretion, indicating that *OsNAAT1* encodes the sole functional enzyme possessing NAAT activity in rice.[15)]

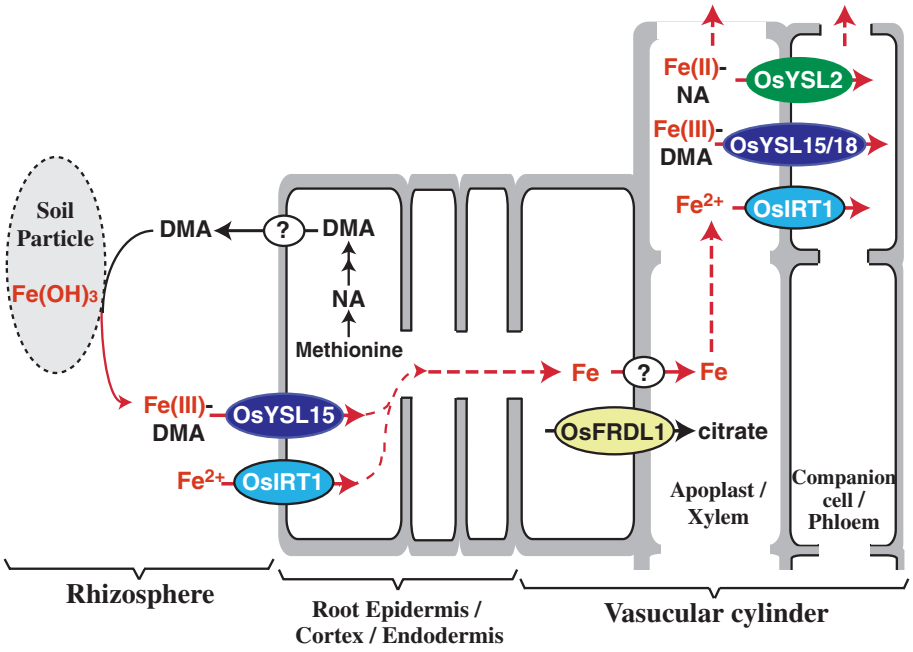

Fig. 2. A simplified scheme of the uptake and translocation of iron in rice. Ovals represent transporters. Iron flow is depicted in red arrows. Xylem loading and phloem unloading of iron would require efflux-type transporters, which are little characterized.

In contrast to the biosynthetic pathway of MAs, the molecular components involved in the secretion of MAs remain unclear. Vesicular transport and the subsequent diurnal secretion of MAs have been suggested.[16)–19)]

MAs secreted to the rhizosphere solubilize Fe(III), and the resulting Fe(III)–MAs complexes are taken up by roots. The gene encoding the Fe(III)–MAs transporter in this iron uptake step, *Yellow Stripe 1* (*YS1*), was first isolated in maize.[20)] Disruption of *YS1* leads to leaf chlorosis due to a defect in Fe(III)–MAs uptake.[21)] Subsequently, a barley homolog of *YS1* (*HvYS1*) was isolated that was shown to transport Fe(III)–MAs.[22)] *HvYS1* is induced in iron deficiency and is localized to root epidermal cells, suggesting its role in uptake. Maize *YS1*, but not barley *HvYS1*, was shown to be localized at the distal side in root epidermal cells.[22),23)]

To identify the rice transporter gene for Fe(III)–DMA uptake, we analyzed the expression of 18 *YS1*-like (*YSL*) genes in rice (*OsYSL1-18*) using microdissected root tissues. Among these genes, *OsYSL15* was strongly up-regulated in the root epidermis under iron-deficient conditions.[24)] *OsYSL15* promoter-*GUS* analysis also revealed strong expression in the epidermal and exodermal cells of iron-deficient roots, where DMA synthesis and the uptake of Fe(III)–DMA complexes is thought to occur.[24)] Furthermore, OsYSL15 transports Fe(III)–DMA in yeast and *Xenopus* oocytes.[24)] Two insertional mutants of *OsYSL15* have been shown to exhibit chlorotic phenotypes in response to iron deficiency.[25)] These results strongly indicate that *OsYSL15* is the rice counterpart of the *YS1/YSL* genes for Fe(III)–DMA uptake (Fig. 2). *HvYS1* and *OsYSL15* expression fluctuates daily,[23),24),26)] possibly so that iron uptake can be coordinated with the diurnal secretion of MAs.[16)] In contrast, the expression of maize *YS1* does not show clear daily fluctuations.[23)]

In non-graminaceous plants, iron uptake from the rhizosphere is mediated by the induction of Fe(III)-chelate reductase and subsequent transport of Fe^{2+} ions across the root plasma membrane (Strategy I).[5)] Eide *et al.*[27)] isolated the *Arabidopsis IRT1* gene, which is the dominant ferrous transporter in this uptake process.[28)] Various transporter genes homologous to *IRT1* have been found in plants, animals, protists, and fungi; they are known collectively as the zinc-regulated transporter, iron-regulated transporter-like protein (ZIP) family.[29)] Rice, in spite of being a Strategy II plant, possesses homologs of the *Arabidopsis IRT1* gene, *OsIRT1* and *OsIRT2*, the ferrous transport capacity of which was demonstrated by functional complementation in yeast.[30),31)] *OsIRT1* expression is strongly induced in iron-

deficient roots, and *OsIRT2* is expressed similarly, but at lower levels. Promoter-*GUS* analysis indicated that *OsIRT1* is expressed mainly in the epidermis, exodermis, and inner layer of the cortex in deficient roots, as well as in the companion cells of shoots. Moreover, analysis using the positron-emitting tracer imaging system (PETIS) revealed that rice is able to take up both Fe(III)–DMA and Fe^{2+}. Thus, rice plants possess a system other than the DMA-based Strategy II for iron uptake (Fig. 2). Such a system to take up Fe^{2+} seems reasonable because rice is commonly grown under submerged conditions in which the dominant form of soil iron is Fe^{2+}.

3. Genes involved in iron translocation

The translocation of iron and other minerals inside the plant body involves a sequence of processes that require various metal chelators and transporters. The chemical properties of iron, including poor solubility and high reactivity, compel plants to use suitable chelating molecules inside their bodies.[1),32)] Physiological and molecular studies have indicated that one of the principal chelators inside the plant body is nicotianamine (NA),[32),33)] which is a biosynthetic precursor of MAs (Fig. 1) synthesized by both graminaceous and non-graminaceous plants. Among the 18 *OsYSL* genes identified, *OsYSL2* expression is strongly induced in iron-deficient leaves.[34)] Electrophysiological analyses of *Xenopus* oocytes showed that OsYSL2 transports Fe(II)–NA and manganese (II) [Mn(II)]–NA, but not Fe(III)–MAs. *OsYSL2* promoter-*GUS* analysis revealed that *OsYSL2* is expressed in root companion cells and the phloem cells of leaves and leaf sheaths, where *OsNAS1-3* are also expressed.[12),34)] *OsYSL2* knockdown rice lines produced using RNA interference (RNAi) accumulated less iron and manganese in shoots and seeds.[35)] These results indicate that OsYSL2 is responsible for the long-distance transport of NA-chelated iron and manganese.

In addition to the central role of NA, various lines of evidence suggest that MAs play a role in the internal distribution of iron in graminaceous plants. Endogenous MAs were detected in the shoots of barley and rice, and the amount of MAs increased dramatically under iron deficiency.[36)] DMA was also detected in rice phloem[37)] and xylem sap.[38)] The genes involved in DMA biosynthesis in rice, *OsNAS1-3*, *OsNAAT1*, and *OsDMAS1*, are co-expressed in phloem companion cells in roots and leaves.[12)–14)] Furthermore, the *OsYSL15* transporter gene is also expressed in the phloem companion cells of roots and leaves, as well as in reproductive organs.[24)]

Recently, we reported another Fe(III)–DMA transporter gene, *OsYSL18*.[39)] *OsYSL18* is expressed in restricted plant parts, including the phloem parenchyma and companion cells at the base of every leaf sheath, suggesting its role in phloem iron transport. Based on physiological studies using tracer elements, the basal part of the shoot was designated as a discrimination center (DC), where phloem and xylem structures are associated and minerals and metabolites accumulate prior to translocation to other plant parts.[40)] Recently, we also reported that iron-52 (^{52}Fe) supplied as ^{52}Fe(III)–DMA to the roots of barley is translocated mainly *via* phloem to the youngest leaves, whereas it is translocated mostly *via* xylem to older leaves, suggesting the importance of iron transfer from xylem to phloem in the DC and/or roots.[41)] *OsYSL18* expression was specific to the region close to the DC. Therefore, phloem loading of Fe(III)–DMA *via* OsYSL18 might be important for iron distribution to leaf sheaths, especially where xylem iron translocation is not fully developed.

Organic acids are also common metal chelators inside the plant body. In particular, Fe(III)–citrate has long been believed to be the dominant form of iron in xylem sap.[42),43)] Since xylem is apoplastic, efflux-type transporters for metals and/or metal-chelator complexes should be needed for long-distance transport in addition to influx-type transporters, including YSLs. FRD3, an *Arabidopsis* transporter of the multidrug and toxin efflux family, has been shown to be involved in citrate efflux into xylem, which is needed for efficient iron transport within the plant.[44)] A *FRD3*-like gene in rice, *OsFRDL1*, is expressed in root pericycle cells adjacent to the protoxylem and metaxylem.[45)] Recently, Yokosho *et al.*[46)] reported the function of OsFRDL1 as a citrate effluxer required for efficient iron translocation. Molecular components known to be involved in iron translocation in rice is summarized in Fig. 2.

We recently developed a highly sensitive quantification method for NA and DMA using liquid chromatography/electrospray ionization time-of-flight mass spectrometry (LC/ESI-TOF-MS).[38),47)] Using this method, comparable amounts of NA, DMA, and iron were detected in rice xylem sap.[38)] Time-course measurement during iron deficiency treatment suggested that Fe(III)–DMA is increasingly important for xylem iron translocation during

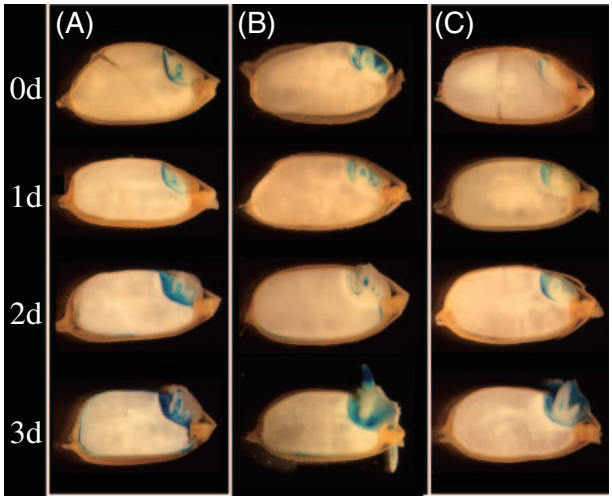

Fig. 3. Localization of transporter gene expression in germinating seeds, as observed by histochemical staining of GUS activity in the *OsYSL2* (A), *OsIRT1* (B) or *OsYSL15* (C) promoter-*GUS* transgenic rice lines in fully mature seeds (0 d) and seeds 1–3 days after sowing.

deficiency, whereas Fe(II)–NA might be the predominant chemical form of iron in xylem sap under iron-sufficient conditions.

Our promoter-*GUS* analysis of rice genes involved in iron acquisition and translocation also revealed that the majority of these genes are expressed in flowers and maturing seeds grown in iron-replete soil, as well as in germinating seeds (Fig. 3).[24),34),39),48)] *OsYSL2*, *OsYSL15*, and *OsYSL18* are also co-expressed in flowers and the vascular bundles of developing seeds.[24),34),39)] Thus, both Fe(II)–NA and Fe(III)–DMA may be involved in seed-iron loading. In addition, *OsYSL18* is strongly expressed in pollen and pollen tubes, suggesting its role in pollen function and fertilization by transporting Fe(III)–DMA into pollen cells.[39)] Notably, *OsYSL15* knockdown seedlings showed a severe arrest in germination and early growth that was rescued by high iron supplementation,[24)] demonstrating the role of OsYSL15 in iron homeostasis during the early stages of growth, facilitating internal Fe(III)–DMA translocation and/or Fe(III)–DMA uptake from the outer surface of seedlings. Recently, synchrotron-based X-ray microfluorescence imaging of iron, zinc, manganese, and copper in rice seeds during germination was carried out at the Super Photon ring-8 GeV (Spring-8) facility.[49)] The distribution of iron in the endosperm and embryo changed during germination, and it differed from that of zinc, manganese, and copper, which may be determined by various expressed metal transporters as suggested by microarray analysis.[49)]

4. Genes involved in the regulation of iron deficiency responses

Under low iron availability, graminaceous plants induce various genes, many of which are involved in iron acquisition and utilization.[2),9),10),50),51)] We identified the novel iron deficiency-responsive *cis*-acting elements IDE1 and IDE2,[52)] which are the first identified elements related to micronutrient deficiencies in plants. IDE1 and IDE2 synergistically induce Fe-deficiency-responsive expression in tobacco roots, as well as in rice roots and leaves.[52),53)] Sequences similar to IDE1 or IDE2 have been found in various Fe deficiency-inducible promoters in barley, rice, tobacco, and *Arabidopsis*.[50),52),54)] This suggests that gene regulatory mechanisms involving IDEs are not only conserved among graminaceous (Strategy II) plants, but are also functional in non-graminaceous (Strategy I) species.

The introduction of graminaceous iron deficiency-responsive promoters into rice, tobacco, and *Arabidopsis* has revealed complicated patterns of compatibility.[52),55)–57)] While the barley *IDS3* promoter confers iron deficiency responses in all three species, the promoters of *HvNAS1* and *OsNAS1* are responsive in rice and tobacco, but not in *Arabidopsis*. The tissue specificities conferred by these promoters are still more complicated, and are possibly dependent on combinations of promoter elements and the presence and/or activation of relevant transcription factors in each tissue. In roots, iron deficiency-induced gene expression is proposed to be mediated by shoot-derived long-distance and local iron signals.[58),59)] Each iron deficiency-induced gene appears to respond to one or both of these signals.[57),60)]

In a recent search for transcription factors that interact with IDEs, we successfully identified two rice transcription factors, IDEF1 (IDE-binding factor 1) and IDEF2, which bind specifically to IDE1 and IDE2, respectively.[61),62)] IDEF1 and IDEF2 belong to uncharacterized branches of the plant-specific transcription factor families ABI3/VP1 and NAC, respectively, and they possess novel properties of sequence recognition. IDEF1 recognizes the CATGC sequence within IDE1, whereas IDEF2 predominantly recognizes CA[A/C]G[T/C][T/C/A][T/C/A] within IDE2 as its core binding site. Northern blotting and histochemical analyses of promoter-*GUS* transgenic rice lines revealed that *IDEF1* and

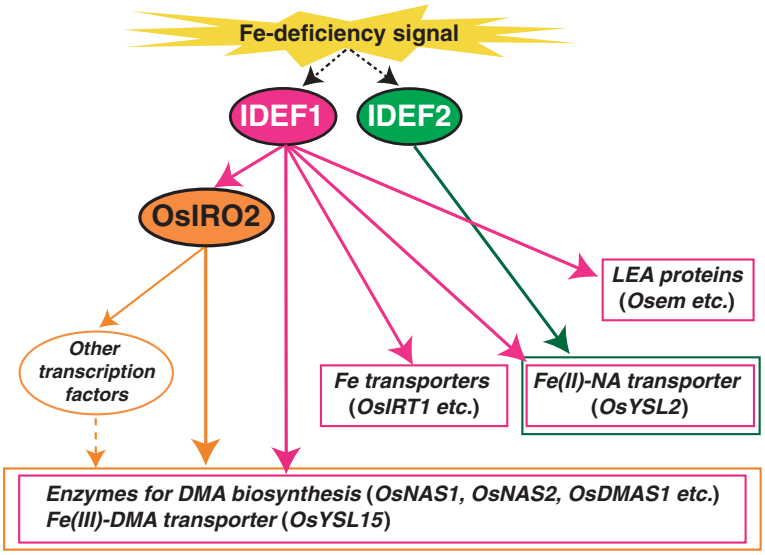

Fig. 4. Model of the gene regulatory network mediated by IDEF1, IDEF2, and OsIRO2 in response to iron deficiency. Dotted lines indicate possible regulation. Ovals indicate transcription factors. All of the indicated genes except *IDEF1* and *IDEF2* are transcriptionally induced in response to iron deficiency.

IDEF2 are constitutively expressed during both vegetative and reproductive growth.[61)–64)]

The characterization of transgenic rice plants with altered *IDEF1* or *IDEF2* expression has revealed the physiological functions of these transcription factors in iron homeostasis. Transgenic rice lines with introduced *IDEF1* under the control of the iron deficiency-inducible *IDS2* promoter exhibited a slower progression of leaf chlorosis in iron-free hydroponic culture and improved early growth when germinated in calcareous soil.[61)] Conversely, *IDEF1* knockdown lines generated by RNAi exhibited hypersensitivity in iron-free hydroponic culture.[63)] During the early stages of iron deficiency, most known iron uptake/utilization-related genes, including *OsIRO2*, *OsIRT1*, *OsYSL15*, *OsYSL2*, *OsNAS1*, *OsNAS2*, *OsNAS3*, and *OsDMAS1*, are positively regulated by IDEF1 (Fig. 4).[63)] In the subsequent stages of iron deficiency, however, the IDEF1-mediated regulation of these iron uptake/utilization-related genes became less obvious. In turn, the expression of several iron deficiency-induced genes encoding late embryogenesis abundant (LEA) proteins, including *Osem* gene, was increasingly regulated by IDEF1 (Fig. 4).[63)] These results and *in silico* *cis*-distribution analysis using microarray data suggest that IDEF1 has a dual function in iron deficiency responses; namely, (i) the coordinated transactivation of iron utilization-related genes *via* CATGC-containing IDE1-like elements, especially at the early stage, and (ii) the transactivation of seed maturation-related genes *via* RY elements, especially during the subsequent stages of iron deficiency.[63),65)]

IDEF2 also regulates iron homeostasis by inducing another subset of deficiency-responsive genes.[62)] *IDEF2* knockdown lines generated by RNAi and IDEF2 dysfunction lines created using Chimeric REpressor Gene-Silencing Technology (CRES-T) exhibit aberrant iron distribution between the roots and shoots, and are defective in the induction of many iron deficiency-responsive genes, including *OsYSL2*.[62)] The regulatory patterns of these IDEF2-dependent genes are largely unaltered between iron sufficiency and early or subsequent deficiency.[64)] The gene regulatory pathways mediated by IDEF1 and IDEF2 are partially, but not predominantly, overlapped.[62),63)] Promoter-*GUS* analysis revealed that *IDEF1* and *IDEF2* are expressed in many plant parts, including root vascular bundles, lateral roots, and leaf blades, as well as in reproductive tissues, including pollen, suggesting their widespread roles.[64)]

To clarify the molecular mechanisms that regulate iron acquisition, we also characterized iron deficiency-induced transcription factors. Microarray analyses revealed that the basic helix-loop-helix (bHLH) transcription factor gene *IRO2* is strongly upregulated by iron deficiency in shoots and roots in barley and rice.[66)] The expression of rice *OsIRO2* (AK073385) is also reportedly induced by gibberellin

in the basal region of rice leaf sheaths.[67] The core sequence for OsIRO2 binding was determined to be CACGTGG.[66] We produced transgenic rice plants with enhanced or repressed *OsIRO2* expression by introducing the *35S-OsIRO2* cassette or using RNAi.[68] In iron-deficient hydroponic culture, *OsIRO2*-overexpressing lines showed enhanced DMA secretion and slightly improved growth compared to non-transformed plants, whereas *OsIRO2*-repressed lines showed reduced DMA secretion and hypersensitivity to iron deficiency. Microarray and Northern blot analyses revealed that the expression of *OsIRO2* is positively related to various iron deficiency-induced genes in roots, including those responsible for DMA biosynthesis (*OsNAS1*, *OsNAS2*, *OsNAAT1*, *OsDMAS1*, and various genes involved in the methionine cycle) and Fe(III)–DMA uptake (*OsYSL15*) (Fig. 4). OsIRO2 also affects the expression of some iron deficiency-inducible transcription factors that possess OsIRO2-binding core sequences in their promoter regions.[68] Importantly, *OsIRO2* itself possesses multiple IDEF1-binding core sequences in its promoter region and is positively regulated by IDEF1.[61] Based on these results, a sequential link in the iron-deficiency response involving IDEF1, IDEF2, OsIRO2, and its downstream iron deficiency-inducible transcription factors is proposed (Fig. 4).[61–63,68]

5. Other mineral elements

Iron transporters and chelators often interact with a wide range of metals other than iron. Thus, iron homeostasis is closely linked to that of other metals, such as zinc, copper, manganese, nickel, and cadmium. Because many of these are essential plant nutrients and all essential and non-essential metals are toxic in excess, the complicated relationships in mineral element translocation are a matter of concern for humans and in agriculture. In particular, zinc deficiency constitutes a major problem in both plant and human nutrition, and cadmium toxicity is a serious problem in human health.

In all plant species, NA is thought to play crucial roles in divalent metal translocation.[32] In addition, enhanced NA production in rice, *Arabidopsis*, and tobacco confers tolerance to excess amounts of various metals, especially nickel,[69,70] indicating its role in protection from metal toxicity.

In graminaceous plants, zinc is thought to be taken up as free zinc (II) [Zn(II)] ions, while the Zn(II)–MAs complex has been proposed as a possible form for uptake.[71,72] Suzuki *et al.*[73] showed that barley induces the expression of MAs biosynthetic genes not only under iron deficiency, but also under zinc deficiency, leading to increased MAs secretion. Moreover, analysis by the PETIS confirmed that more Zn(II)–MAs than Zn^{2+} were absorbed by zinc-deficient barley roots. In contrast to barley, rice does not increase DMA secretion under zinc-deficient conditions.[74] However, Zn(II)–DMA is translocated to zinc-deficient rice leaves at a higher rate than Zn^{2+}, suggesting that zinc is also transported internally in an DMA-chelated form.[74] In contrast, Meda *et al.*[75] reported that chelate formation of cadmium (II) [Cd(II)]–DMA is much weaker than that of Fe(III)–DMA and Zn(II)–DMA, and that DMA does not function in cadmium uptake. DMA-mediated iron acquisition was suggested to be advantageous for improving iron uptake in the presence of cadmium.

ZIP transporters in various organisms transport not only Fe^{2+}, but also other divalent cations, including Zn^{2+}, Mn^{2+}, and Cd^{2+}.[6] Among the rice ZIP members, *OsZIP4* expression is strongly induced under zinc deficiency.[76] Along with its Zn^{2+}-transporting capacity, *OsZIP4* expression in vascular tissues, leaf mesophyll cells, and apical meristems suggests its predominant role in zinc translocation and utilization in rice. Overexpression of *OsZIP4* under the control of the constitutive *35S* promoter leads to zinc accumulation in roots and severe zinc deficiency in shoots, suggesting that *OsZIP4* needs to be strictly regulated.[77]

The barley ortholog of IRT1, HvIRT1, was reported to transport Fe^{2+}, Mn^{2+}, Zn^{2+}, and Cd^{2+}.[78] Both iron and manganese deficiencies induce *HvIRT1* expression, and increased expression of *HvIRT1* in a manganese-efficient genotype with a higher Mn^{2+} uptake rate suggests the contribution of HvIRT1 to manganese uptake in barley.[78] On the other hand, OsIRT1 and OsIRT2 do not complement yeast growth defective in Zn^{2+}, Mn^{2+}, or Cu^{2+} uptake.[31] However, these transporters enhance yeast sensitivity to Cd^{2+},[79] suggesting their ability to transport Cd^{2+}, and thus may constitute a substantial influx route of cadmium into rice grains.

Recently, we performed a microarray analysis of rice following cadmium treatment.[80] Many genes responded to short- and/or long-term cadmium exposure. Genes involved in DMA biosynthesis were induced in the roots following long-term cadmium treatment, suggesting induced iron deficiency by cadmium stress. Similarly, a microarray analysis of rice plants treated with excess zinc revealed the

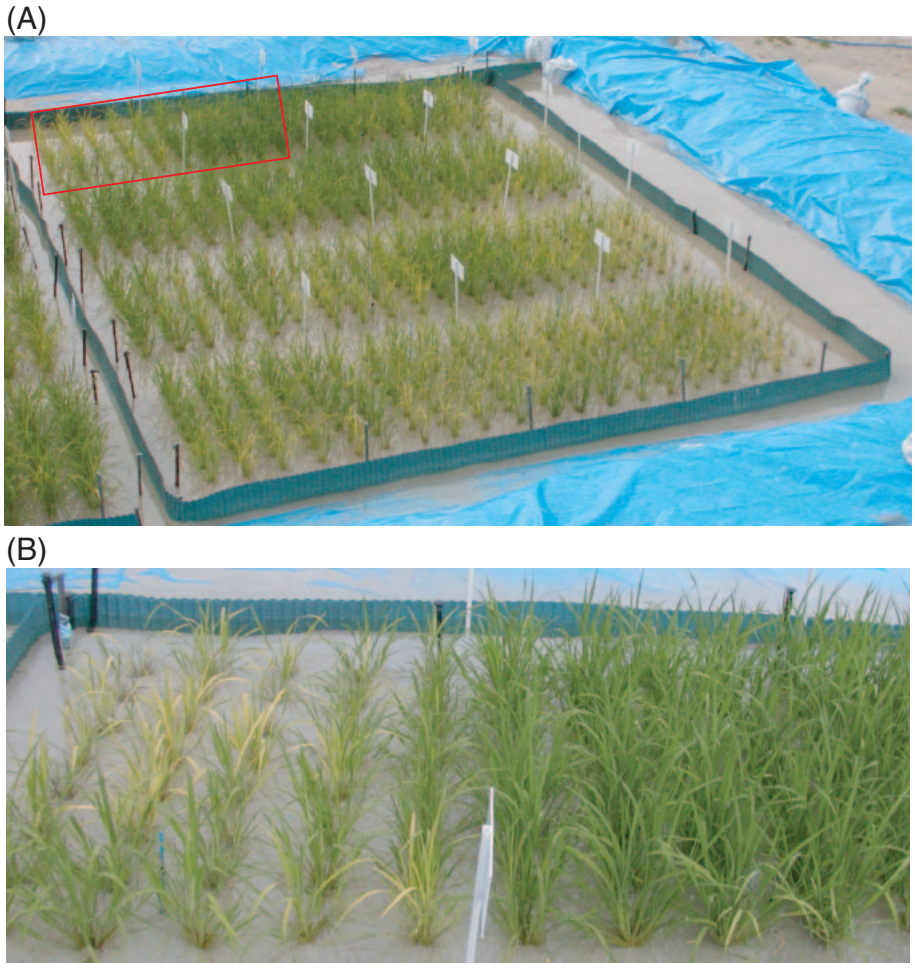

Fig. 5. Iron deficiency tolerance of transgenic rice plants in a calcareous paddy field. (A) Photograph of the entire field at 35 days after transplanting. The rice lines tested are those transformed with barley genome fragment(s) containing (i) *IDS3*, (ii) *HvNAS1*, and (iii) *HvNAS1*, *HvNAAT-A* and *-B*, as well as non-transformants. (B) Visual comparison between *IDS3*-transformed line (right) and non-transformants (left) at 44 days after transplanting, as illustrated in the red box in (A).

induction of DMA biosynthetic genes.[81] However, induced expression of *OsIRO2*, but not *OsIRT1*, in the presence of excess zinc, suggested the presence of a pathway that induces OsIRO2-mediated DMA biosynthesis in zinc-overtreated plants, which is not simply caused by iron deficiency.

6. Production of transgenic crops with improved nutritional traits

Iron deficiency tolerance is divergent among graminaceous plants and is thought to be dependent on the amount and types of MAs that they secrete. Rice, sorghum, and maize secrete only small amounts of DMA among the MAs, and thus are susceptible to low iron availability. We have long aimed to produce transgenic crops, especially rice, with enhanced tolerance to iron deficiency (reviewed by Ref. 10). This was first achieved by introducing a genome fragment containing barley *HvNAAT-A* and *HvNAAT-B* into rice.[82] These transgenic lines secreted an increased amount of MAs and exhibited substantial tolerance to calcareous soils. Subsequently, various rice lines carrying one or more barley genes for MAs biosynthesis have been produced, some of which have been subjected to field experiments in calcareous soil under paddy conditions (Fig. 5).[83] Two rice lines, one with a genome fragment containing barley NA synthase gene *HvNAS1* and another with a barley genome fragment of mugineic acid synthase gene *IDS3*, exhibited tolerance to low iron availability. Thus, a transgenic approach to increasing the tolerance of rice to low

iron availability is practical for improving agricultural productivity in calcareous soils.

Another effective approach to improving iron deficiency tolerance was accomplished by introducing ferric-chelate reductase activity in rice.[84] Although rice is able to take up Fe^{2+} *via* the OsIRT1 transporter, its ferric-chelate reductase activity is low and not induced in response to iron deficiency.[31] Therefore, we introduced a reconstructed and mutagenized derivative of the yeast ferric reductase gene *FRE1*, designated *refre1/372*,[85] which has been artificially evolved to possess enhanced enzymatic activity at a high pH for preferential function in calcareous soils. We used the *OsIRT1* promoter to drive the expression of *refre1/372*, thereby enabling the expression of ferric-chelate reductase and ferrous transporter in the same cells under the same conditions.[84] Transgenic rice plants with the introduced *OsIRT1* promoter-*refre1/372* construct successfully induced ferric-chelate reductase expression and activity in iron-deficient roots, leading to increased iron uptake compared to the controls, as revealed by the PETIS. The transformants exhibited enhanced tolerance to low iron availability in both hydroponic culture and calcareous soil. When grown in calcareous soil until harvest, the transformants yielded 7.9 times more grain than the vector controls, demonstrating that creating a complete Strategy I system in rice by enhancing ferric-chelate reductase activity is effective in improving iron-deficiency tolerance.

As mentioned above, the manipulation of transcription factors regulating iron homeostasis could be another potent method for enhancing deficiency tolerance. We recently found that the overexpression of *OsIRO2* was much more effective than *IDEF1* induction in conferring long-term iron deficiency tolerance to rice plants grown in calcareous soils (unpublished results). When grown in a calcareous soil until maturation, *35S* promoter-*OsIRO2* transformants grew better than non-transformants, resulting in higher grain yields.[86]

Understanding iron homeostasis also paves the way for fortifying rice grains with iron and zinc, which would greatly contribute to human nutrition. Field trials revealed that a rice line carrying a barley *IDS3* genome fragment was not only tolerant to low iron availability in calcareous soils, but was also capable of accumulating more zinc and iron in its grains in both calcareous and Andosol paddy fields.[83),87] Because hydroxylated MAs are more stable under mildly acidic conditions,[88] MAs synthesized by IDS3 would be favorable for the internal translocation of iron and zinc. Furthermore, the overexpression of barley *HvNAS1* under the control of either the *35S* or *OsActin1* promoter resulted in three- and two-fold increases in the iron and zinc concentrations, respectively, in polished T_1 grains as compared to non-transformants.[89] Similarly, activation-tagged *OsNAS3* overexpression rice lines showed three- and two-fold increases in iron and zinc, respectively, in their grains.[70] In these transgenic rice lines, the amounts of NA and DMA were increased.[70),89] These results indicate that enhanced NA and DMA production results in the efficient translocation of iron and zinc to rice grains. Further, Lee *et al.*[70] showed that increased iron became incorporated into a low molecular mass of possible Fe–NA cluster, which was effective in alleviating anemic symptoms in mice. More recently, we produced transgenic rice lines with iron concentrations that were increased up to 4.4-fold in their polished seeds by expressing *OsYSL2* under the control of a sucrose transporter (*OsSUT1*) promoter.[35] The overexpression of *OsIRT1* in rice also led to slight increases in iron and zinc accumulation in grains.[90]

Nutrient accumulation in grains is achieved by the combined effects of uptake from the soil and translocation from source organs. Uauy *et al.*[91] reported that the ancestral wild wheat allele encodes a NAC transcription factor (NAM-B1) that accelerates senescence and increases the remobilization of nutrients from leaves to developing grains. Knockdown of the corresponding gene delayed senescence and reduced the wheat grain protein, iron, and zinc contents.[91),92] Although rice counterparts of this gene have not been identified, another rice *NAC* gene, *OsNAC5*, is up-regulated in flag leaves during grain filling and might be involved in the remobilization of iron, zinc, and amino acids to grains.[93] *OsNAC5* expression in leaves is regulated by abscisic acid[93] and is induced under iron deficiency in some cases,[66] but not in others.[93]

Since the report of the overexpression of ferritin, a common iron storage protein, in rice grains by Goto *et al.*,[94] biofortification of rice seeds with iron using ferritin has been attempted in many laboratories worldwide. Wirth *et al.*[95] recently reported a greater than six-fold increase in iron concentration in rice endosperm following the combined introduction of the *Arabidopsis NAS* gene *AtNAS1* driven by the *35S* promoter and *Pvferritin* driven by the rice seed storage globulin promoter. Transgenic rice lines

produced recently by our group carrying various combinations of iron homeostasis/storage-related genes driven by several tissue-specific promoters have been shown to possess still greater polished seed concentrations of iron and zinc (unpublished data).

7. Future perspectives

Based on the fundamental knowledge accumulated during continuous studies of graminaceous plant responses to iron deficiency and recent advances in genome information, much progress has been made in recent years on iron homeostasis in rice. Many crucial genes for iron translocation and regulation have been identified and characterized. Other recent findings include the induction of glutathione reductase expression and activity in iron-deficient graminaceous plants,[96] suggesting a close relationship between iron homeostasis and cellular redox status. We identified a rice-specific mitochondrial iron deficiency-induced gene (*MIR*), whose knockout severely impairs rice growth.[97] Mechanisms of subcellular compartmentalization for iron and other mineral elements are poorly understood, especially in graminaceous plants. Also, mechanisms of iron deficiency sensing and signaling, as well as the secretory mechanism of MAs and NA into apoplastic spaces, have not been clarified. A comprehensive understanding of the speciation of metal chelates and transporters in each step of metal translocation will become increasingly important for future advances in this field and for the production of crops with improved nutritional traits, including those with low cadmium in the edible parts and high cadmium plants applicable for phytoremediation.

Acknowledgements

We thank Dr. Motofumi Suzuki (Aichi Steel Co.) and Dr. Kendi Claudio Morikawa (National Institute of Vegetable and Tea Science) for providing us with the pictures of rice field trial. This research was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN) and a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Genomics for Agricultural Innovation, GMB0001).

References

1) Marschner, H. (1995) Mineral Nutrition of Higher Plants 2nd ed. Academic press, London, UK.
2) Mori, S. (1999) Iron acquisition by plants. Curr. Opin. Plant Biol. **2**, 250–253.
3) Grusak, M.A. and Dellapenna, D. (1999) Improving the nutrient composition of plants to enhance human nutrition and health. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**, 133–161.
4) Takagi, S. (1976) Naturally occurring iron-chelating compounds in oat- and rice-root washing. I. Activity measurement and preliminary characterization. Soil Sci. Plant Nutr. **22**, 423–433.
5) Römheld, V. and Marschner, H. (1986) Evidence for a specific uptake system for iron phytosiderophore in roots of grasses. Plant Physiol. **80**, 175–180.
6) Morrissey, J. and Guerinot, M.L. (2009) Iron uptake and transport in plants: the good, the bad, and the ionome. Chem. Rev. **109**, 4553–4567.
7) Palmer, C.M. and Guerinot, M.L. (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat. Chem. Biol. **5**, 333–340.
8) Pilon, M., Cohu, C.M., Ravet, C., Abdel-Ghany, S.E. and Gaymard, F. (2009) Essential transition metal homeostasis in plants. Curr. Opin. Plant Biol. **12**, 347–357.
9) Kobayashi, T., Nishizawa, N.K. and Mori, S. (2006) Molecular analysis of iron-deficient Graminaceous plants. *In* Iron Nutrition in Plants and Rhizospheric Microorganisms (eds. Barton, L.L. and Abadía, J.). Springer Inc., pp. 395–435.
10) Kobayashi, T., Nakanishi, H., Takahashi, M., Mori, S. and Nishizawa, N.K. (2008) Generation and field trials of transgenic rice tolerant to iron deficiency. Rice **1**, 144–153.
11) Ueno, D., Rombolà, A.D., Iwashita, T., Nomoto, K. and Ma, J.F. (2007) Identification of two new phytosiderophores secreted by perennial grasses. New Phytol. **174**, 304–310.
12) Inoue, H., Higuchi, K., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2003) Three rice nicotianamine synthase genes, *OsNAS1, OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. Plant J. **36**, 366–381.
13) Inoue, H., Takahashi, M., Kobayashi, T., Suzuki, M., Nakanishi, H., Mori, S. *et al.* (2008) Identification and localisation of the rice nicotianamine aminotransferase gene *OsNAAT1* expression suggests the site of phytosiderophore synthesis in rice. Plant Mol. Biol. **66**, 193–203.
14) Bashir, K., Inoue, H., Nagasaka, S., Takahashi, M., Nakanishi, H., Mori, S. *et al.* (2006) Cloning and characterization of deoxymugineic acid synthase genes from graminaceous plants. J. Biol. Chem. **43**, 32395–32402.
15) Cheng, L., Wang, F., Shou, H., Huang, F., Zheng, L., He, F. *et al.* (2007) Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. Plant

Physiol. **145**, 1647–1657.
16) Takagi, S., Nomoto, K. and Takemoto, S. (1984) Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. J. Plant Nutr. **7**, 469–477.
17) Nishizawa, N. and Mori, S. (1987) The particular vesicle appearing in barley root cells and its relation to mugineic acid secretion. J. Plant Nutr. **10**, 1013–1020.
18) Negishi, T., Nakanishi, H., Yazaki, J., Kishimoto, N., Fujii, F., Shimbo, K. *et al.* (2002) cDNA microarray analysis of gene expression during Fe-deficiency stress in barley suggests that polar transport of vesicles is implicated in phytosiderophore secretion in Fe-deficient barley roots. Plant J. **30**, 83–94.
19) Nozoye, T., Itai, R.N., Nagasaka, S., Takahashi, M., Nakanishi, H., Mori, S. *et al.* (2004) Diurnal changes in the expression of genes that participate in phytosiderophore synthesis in rice. Soil Sci. Plant Nutr. **50**, 1125–1131.
20) Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S.L., Briat, J.F. and Walker, E.L. (2001) Maize *yellow stripe 1* encodes a membrane protein directly involved in Fe(III) uptake. Nature **409**, 346–349.
21) von Wirén, N., Mori, S., Marschner, H. and Römheld, V. (1994) Iron inefficiency in maize mutant ys1 (*Zea mays* L. cv yellow-stripe) is caused by a defect in uptake of iron phytosiderophores. Plant Physiol. **106**, 71–77.
22) Murata, Y., Ma, J.F., Yamaji, N., Ueno, D., Nomoto, K. and Iwashita, T. (2006) A specific transporter for iron(III)-phytosiderophore in barley roots. Plant J. **46**, 563–572.
23) Ueno, D., Yamaji, N. and Ma, J.F. (2009) Further characterization of ferric-phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley. J. Exp. Bot. **60**, 3513–3520.
24) Inoue, H., Kobayashi, T., Nozoye, T., Takahashi, M., Kakei, Y., Suzuki, K. *et al.* (2009) Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J. Biol. Chem. **284**, 3470–3479.
25) Lee, S., Chiecko, J.C., Kim, S.A., Walker, E.L., Lee, Y., Guerinot, M.L. *et al.* (2009) Disruption of *OsYSL15* leads to iron inefficiency in rice plants. Plant Physiol. **150**, 786–800.
26) Nagasaka, S., Takahashi, M., Itai, R.N., Bashir, K., Nakanishi, H., Mori, S. *et al.* (2009) Time course analysis of gene expression over 24 hours in Fe-deficient barley roots. Plant Mol. Biol. **69**, 621–631.
27) Eide, D., Broderius, M., Fett, J. and Guerinot, M.L. (1996) A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc. Natl. Acad. Sci. USA **93**, 5624–5628.
28) Vert, G., Grotz, N., Dedaldechamp, F., Gaymard, F., Guerinot, M.L., Briat, J.F. *et al.* (2002) IRT1, an Arabidopsis transporter essential for iron uptake from the soil and plant growth. Plant Cell **14**, 1223–1233.
29) Guerinot, M.L. (2000) The ZIP family of metal transporters. Biochim. Biophys. Acta **1465**, 190–198.
30) Bughio, N., Yamaguchi, H., Nishizawa, N.K., Nakanishi, H. and Mori, S. (2002) Cloning of iron-regulated metal transporter from rice. J. Exp. Bot. **53**, 1677–1682.
31) Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T. *et al.* (2006) Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J. **45**, 335–346.
32) Hell, R. and Stephan, U.W. (2003) Iron uptake, trafficking and homeostasis in plants. Planta **216**, 541–551.
33) Takahashi, M., Terada, Y., Nakai, I., Nakanishi, H., Yoshimura, E., Mori, S. *et al.* (2003) Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell **15**, 1263–1280.
34) Koike, S., Inoue, H., Mizuno, D., Takahashi, M., Nakanishi, H., Mori, S. *et al.* (2004) *OsYSL2* is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. Plant J. **39**, 415–424.
35) Ishimaru, Y., Masuda, H., Bashir, K., Inoue, H., Tsukamoto, T., Takahashi, M. *et al.* (2010) Rice metal-nicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. Plant J. **62**, 379–390.
36) Higuchi, K., Watanabe, S., Takahashi, M., Kawasaki, S., Nakanishi, H., Nishizawa, N.K. *et al.* (2001) Nicotianamine synthase gene expression differs in barley and rice under Fe-deficient conditions. Plant J. **25**, 159–167.
37) Mori, S., Nishizawa, N., Hayashi, H., Chino, M., Yoshimura, E. and Ishihara, J. (1991) Why are young rice plants highly susceptible to iron deficiency? Plant Soil **130**, 143–156.
38) Kakei, Y., Yamaguchi, I., Kobayashi, T., Takahashi, M., Nakanishi, H., Yamakawa, T. *et al.* (2009) A highly sensitive, quick, and simple quantification method for nicotianamine and 2′-deoxymugineic acid from minimum samples using LC/ESI-TOF-MS achieves functional analysis of these components in plants. Plant Cell Physiol. **50**, 1988–1993.
39) Aoyama, T., Kobayashi, T., Takahashi, M., Nagasaka, S., Usuda, K., Kakei, Y. *et al.* (2009) OsYSL18 is a rice iron(III)–deoxymugineic acid transporter specifically expressed in reproductive organs and phloem of lamina joints. Plant Mol. Biol. **70**, 681–692.
40) Kiyomiya, S., Nakanishi, H., Uchida, H., Tsuji, A., Nishiyama, S., Futatsubashi, M. *et al.* (2001) Real time visualization of ^{13}N-translocation in rice under different environmental conditions using a positron-emitting tracer imaging system. Plant Physiol. **125**, 1743–1754.
41) Tsukamoto, T., Nakanishi, H., Uchida, H., Watanabe, S., Matsuhashi, S., Mori, S. *et al.* (2009) ^{52}Fe translocation in barley as monitored by a positron emitting tracer imaging system

(PETIS): evidence for the direct translocation of Fe from roots to young leaves via phloem. Plant Cell Physiol. **50**, 48–57.
42) Tiffin, L.O. (1966) Iron translocation: II. Citrate/iron ratios in plant stem exudates. Plant Physiol. **41**, 515–518.
43) Brown, J.C. and Chaney, R.L. (1971) Effect of iron on the transport of citrate into the xylem of soybean and tomatoes. Plant Physiol. **47**, 836–840.
44) Durrett, T.P., Gassmann, W. and Rogers, E.E. (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiol. **144**, 197–205.
45) Inoue, H., Suzuki, M., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2004) A rice FRD3-like (OsFRDL1) gene is expressed in the cells involved in long-distance transport. Soil Sci. Plant Nutr. **50**, 1133–1140.
46) Yokosho, K., Yamaji, N., Ueno, D., Mitani, N. and Ma, J.F. (2009) OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. Plant Physiol. **149**, 297–305.
47) Wada, Y., Yamaguchi, I., Takahashi, M., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2007) Highly sensitive quantitative analysis of nicotianamine using LC/ESI-TOF-MS with an internal standard. Biosci. Biotechnol. Biochem. **71**, 435–441.
48) Nozoye, T., Inoue, H., Takahashi, M., Ishimaru, Y., Nakanishi, H., Mori, S. *et al.* (2007) The expression of iron homeostasis-related genes during rice germination. Plant Mol. Biol. **64**, 35–47.
49) Takahashi, M., Nozoye, T., Kitajima, N., Fukuda, N., Hokura, A., Terada, Y. *et al.* (2009) In vivo analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray fluorescence imaging of Fe, Zn, Mn, and Cu. Plant Soil **325**, 39–51.
50) Kobayashi, T., Suzuki, M., Inoue, H., Itai, R.N., Takahashi, M., Nakanishi, H. *et al.* (2005) Expression of iron-acquisition-related genes in iron-deficient rice is co-ordinately induced by partially conserved iron-deficiency-responsive elements. J. Exp. Bot. **56**, 1305–1316.
51) Kobayashi, T. and Nishizawa, N.K. (2008) Regulation of iron and zinc uptake and translocation in rice. *In* Rice Biology in the Genomics Era (eds. Hirano, H.Y., Hirai, A., Sano, Y. and Sasaki, T.). Springer Inc., pp. 321–335.
52) Kobayashi, T., Nakayama, Y., Itai, R.N., Nakanishi, H., Yoshihara, T., Mori, S. *et al.* (2003) Identification of novel *cis*-acting elements, IDE1 and IDE2, of the barley *IDS2* gene promoter conferring iron-deficiency-inducible, root-specific expression in heterogeneous tobacco plants. Plant J. **36**, 780–793.
53) Kobayashi, T., Nakayama, Y., Takahashi, M., Inoue, H., Nakanishi, H., Yoshihara, T. *et al.* (2004) Construction of artificial promoters highly responsive to iron deficiency. Soil Sci. Plant Nutr. **50**, 1167–1175.
54) Ducos, E., Fraysse, Å.S. and Boutry, M. (2005) *NtPDR3*, an iron-deficiency inducible ABC transporter in *Nicotiana tabacum*. FEBS Lett. **579**, 6791–6795.
55) Kobayashi, T., Yoshihara, T., Itai, R.N., Nakanishi, H., Takahashi, M., Mori, S. *et al.* (2007) Promoter analysis of iron-deficiency-inducible barley *IDS3* gene in *Arabidopsis* and tobacco plants. Plant Physiol. Biochem. **45**, 262–269.
56) Ito, S., Inoue, H., Kobayashi, T., Yoshiba, M., Mori, S., Nishizawa, N.K. *et al.* (2007) Interspecies compatibility of the *NAS1* gene promoters. Plant Physiol. Biochem. **45**, 270–276.
57) Ito, S., Inoue, H., Kobayashi, T., Yoshiba, M., Mori, S., Nishizawa, N.K. *et al.* (2009) Comparison of the functions of the barley nicotianamine synthase gene *HvNAS1* and rice nicotianamine synthase gene *OsNAS1* promoters in response to iron deficiency in transgenic tobacco. Soil Sci. Plant Nutr. **55**, 277–282.
58) Enomoto, Y., Hodoshima, H., Shimada, H., Shoji, K., Yoshihara, T. and Goto, F. (2007) Long-distance signals positively regulate the expression of iron uptake genes in tobacco roots. Planta **227**, 81–89.
59) Giehl, R.F.H., Meda, A.R. and von Wirén, N. (2009) Moving up, down, and everywhere: signaling of micronutrients in plants. Curr. Opin. Plant Biol. **12**, 320–327.
60) Enomoto, Y., Hashida, S., Shoji, K., Shimada, H., Yoshihara, T. and Goto, F. (2009) Expressions of iron uptake genes in roots are affected by long-distance signals both in non-graminaceous and in graminaceous plants. *In* Proceedings of XVI International Plant Nutrition Colloquium. Paper 1209.
61) Kobayashi, T., Ogo, Y., Itai, R.N., Nakanishi, H., Takahashi, M., Mori, S. *et al.* (2007) The transcription factor IDEF1 regulates the response to and tolerance of iron deficiency in plants. Proc. Natl. Acad. Sci. USA **104**, 19150–19155.
62) Ogo, Y., Kobayashi, T., Itai, R.N., Nakanishi, H., Kakei, Y., Takahashi, M. *et al.* (2008) A novel NAC transcription factor IDEF2 that recognizes the iron deficiency-responsive element 2 regulates the genes involved in iron homeostasis in plants. J. Biol. Chem. **283**, 13407–13417.
63) Kobayashi, T., Itai, R.N., Ogo, Y., Kakei, Y., Nakanishi, H., Takahashi, M. *et al.* (2009) The rice transcription factor IDEF1 is essential for the early response to iron deficiency, and induces vegetative expression of late embryogenesis abundant genes. Plant J. **60**, 948–961.
64) Kobayashi, T., Ogo, Y., Aung, M.S., Nozoye, T., Itai, R.N., Nakanishi, H. *et al.* (2010) The spatial expression and regulation of transcription factors IDEF1 and IDEF2. Ann. Bot. (Lond.) **105**, 1109–1117.
65) Kobayashi, T., Nakanishi, H. and Nishizawa, N.K. (2010) Dual regulation of iron deficiency response mediated by the transcription factor IDEF1. Plant Signal. Behav. **5**, 157–159.
66) Ogo, Y., Itai, R.N., Nakanishi, H., Inoue, H., Kobayashi, T., Suzuki, M. *et al.* (2006) Isolation

and characterization of IRO2, a novel iron-regulated bHLH transcription factor in graminaceous plants. J. Exp. Bot. **57**, 2867–2878.
67) Komatsu, S. and Takasaki, H. (2009) Gibberellin-regulated gene in the basal region of rice leaf sheath encodes basic helix-loop-helix transcription factor. Amino Acids **37**, 231–238.
68) Ogo, Y., Itai, R.N., Nakanishi, H., Kobayashi, T., Takahashi, M., Mori, S. *et al.* (2007) The rice bHLH protein OsIRO2 is an essential regulator of the genes involved in Fe uptake under Fe-deficient conditions. Plant J. **51**, 366–377.
69) Kim, S., Takahashi, M., Higuchi, K., Tsunoda, K., Nakanishi, H., Yoshimura, E. *et al.* (2005) Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiol. **46**, 1809–1818.
70) Lee, S., Jeon, U.S., Lee, S.J., Kim, Y.-K., Persson, D.P., Husted, S. *et al.* (2009) iron fortification of rice seeds through activation of the *nicotianamine synthase* gene. Proc. Natl. Acad. Sci. USA **106**, 22014–22019.
71) Welch, R.M. (1995) Micronutrient nutrition of plants. Crit. Rev. Plant Sci. **14**, 49–82.
72) von Wirén, N., Marschner, H. and Römheld, V. (1996) Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. Plant Physiol. **111**, 1119–1125.
73) Suzuki, M., Takahashi, M., Tsukamoto, T., Watanabe, S., Matsuhashi, S., Yazaki, J. *et al.* (2006) Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. Plant J. **48**, 85–97.
74) Suzuki, M., Tsukamoto, T., Inoue, H., Watanabe, S., Matsuhashi, S., Takahashi, M. *et al.* (2008) Deoxymugineic acid increases Zn translocation in Zn-deficient rice plants. Plant Mol. Biol. **66**, 609–617.
75) Meda, A.R., Scheuermann, E.B., Prechsl, U.E., Erenoglu, B., Schaaf, G., Hayen, H. *et al.* (2007) Iron acquisition by phytosiderophores contributes to cadmium tolerance. Plant Physiol. **143**, 1761–1773.
76) Ishimaru, Y., Suzuki, M., Kobayashi, T., Takahashi, M., Nakanishi, H., Mori, S. *et al.* (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J. Exp. Bot. **56**, 3207–3214.
77) Ishimaru, Y., Masuda, H., Suzuki, M., Bashir, K., Takahashi, M., Nakanishi, H. *et al.* (2007) Overexpression of the OsZIP4 zinc transporter confers disarrangement of zinc distribution in rice plants. J. Exp. Bot. **58**, 2909–2915.
78) Pedas, P., Ytting, C.K., Fuglsang, A.T., Jahn, T.P., Schjoerring, J.K. and Husted, S. (2008) Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. Plant Physiol. **148**, 455–466.
79) Nakanishi, H., Ogawa, I., Ishimaru, Y., Mori, S. and Nishizawa, N.K. (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe^{2+} transporters OsIRT1 and OsIRT2 in rice. Soil Sci. Plant Nutr. **52**, 464–469.
80) Ogawa, I., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2009) Time course analysis of gene regulation under cadmium stress in rice. Plant Soil **325**, 97–108.
81) Ishimaru, Y., Suzuki, M., Ogo, Y., Takahashi, M., Nakanishi, H., Mori, S. *et al.* (2008) Synthesis of nicotianamine and deoxymugineic acid is regulated by OsIRO2 in Zn excess rice plants. Soil Sci. Plant Nutr. **54**, 417–423.
82) Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N.K. and Mori, S. (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nat. Biotechnol. **19**, 466–469.
83) Suzuki, M., Morikawa, K.C., Nakanishi, H., Takahashi, M., Saigusa, M., Mori, S. *et al.* (2008) Transgenic rice lines that include barley genes have increased tolerance to low iron availability in a calcareous paddy soil. Soil Sci. Plant Nutr. **54**, 77–85.
84) Ishimaru, Y., Kim, S., Tsukamoto, T., Oki, H., Kobayashi, T., Watanabe, S. *et al.* (2007) Mutational reconstructed ferric chelate reductase confers enhanced tolerance in rice to iron deficiency in calcareous soil. Proc. Natl. Acad. Sci. USA **104**, 7373–7378.
85) Oki, H., Kim, S., Nakanishi, H., Takahashi, M., Yamaguchi, H., Mori, S. *et al.* (2004) Directed evolution of yeast ferric reductase to produce plants with tolerance to iron deficiency in alkaline soils. Soil Sci. Plant Nutr. **50**, 1159–1165.
86) Ogo, Y., Itai, R.N., Kobayashi, T., Nakanishi, H., Mori, S. and Nishizawa, N.K. (2009) Overexpression of *OsIRO2* improves both iron uptake and translocation to seeds in rice. *In* Proceedings of XVI International Plant Nutrition Colloquium. Paper 1204.
87) Masuda, H., Suzuki, M., Morikawa, K.C., Kobayashi, T., Nakanishi, H., Takahashi, M. *et al.* (2008) Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. Rice **1**, 100–108.
88) von Wirén, N., Khodr, H. and Hider, R.C. (2000) Hydroxylated phytosiderophore species from rye and barley possess an enhanced chelating efficiency and affinity for iron(III). Plant Physiol. **124**, 1149–1157.
89) Masuda, H., Usuda, K., Kobayashi, T., Ishimaru, Y., Kakei, Y., Takahashi, M. *et al.* (2009) Overexpression of the barley nicotianamine synthase gene HvNAS1 increases iron and zinc concentrations in rice grains. Rice **2**, 155–166.
90) Lee, S. and An, G. (2009) Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. Plant Cell Environ. **32**, 408–416.
91) Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. and Dubcovsky, J. (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science **314**, 1298–1301.
92) Waters, B.M., Uauy, C., Dubcovsky, J. and Grusak,

M. (2009) Wheat (Triticum aestivum) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. J. Exp. Bot. **60**, 4263–4274.
93) Sperotto, R.A., Ricachenevsky, F.K., Duarte, G.L., Boff, T., Lopes, K.L., Sperb, E.R. *et al.* (2009) Identification of up-regulated genes in flag leaves during rice grain filling and characterization of *Os*NAC5, a new ABA-dependent transcription factor. Planta **230**, 985–1002.
94) Goto, F., Yoshihara, T., Shigemoto, N., Toki, S. and Takaiwa, F. (1999) Iron fortificaton of rice seed by the soybean ferritin gene. Nat. Biotechnol. **17**, 282–286.
95) Wirth, J., Poletti, S., Aeschlimann, B., Yakandawala, N., Drosse, B., Osorio, S. *et al.* (2009) Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. Plant Biotechnol. J. **7**, 1–14.
96) Bashir, K., Nagasaka, S., Itai, R.N., Kobayashi, T., Takahashi, M., Nakanishi, H. *et al.* (2007) Expression and enzyme activity of glutathione reductase is upregulated by Fe-deficiency in graminaceous plants. Plant Mol. Biol. **65**, 277–284.
97) Ishimaru, Y., Bashir, K., Fujimoto, M., An, G., Itai, R.N., Tsutsumi, N. *et al.* (2009) Rice-specific mitochondrial iron-regulated gene (MIR) plays an important role in iron homeostasis. Mol. Plant **2**, 1059–1066.

(Received Apr. 2, 2010; accepted Sept. 27, 2010)

Profile

Naoko K Nishizawa was born in 1945. She earned her bachelor's degree in Agricultural Chemistry, Faculty of Agriculture at the University of Tokyo in 1968 and her Ph. D. in Agricultural Sciences from the University of Tokyo. She started her career with studies on the ultra-structural analysis of crop plants and performed pioneering work on crop uptake of organic compounds and found that rice plants uptake and utilize organic nitrogen as a nutrient source by the mechanism of heterophagy in 1977. This was the first demonstration of the occurrence of heterophagy mechanism in the plants. She employed as an assistant professor at the University of Tokyo in 1982. She and Dr. Satoshi Mori started to work on iron acquisition mechanism in graminaceous plants in collaboration with Prof. Seiichi Takagi who found mugineic family phytosiderophores, and clarified the biosynthetic pathway of phytosiderophores. This study led to subsequent extensive studies that identified many proteins and genes participated in iron acquisition and their regulatory network, and developed the transgenic rice with enhanced tolerance to iron deficiency in calcareous soil. From 1995 to 1996, she was in the laboratory of plant molecular biology of The Rockefeller University and working on the molecular mechanism of signal transduction pathway in plants. She was promoted to a lecturer in 1996 and to full professor in 1997. She moved to Research Institute for Bioresources and Biotechnology at Ishikawa Prefectural University as full professor in 2009. She is an elected member of Science Council of Japan and was awarded the Japan Prize of Agricultural Sciences in 2010.