



OsFPN1 functions as an essential metal transporter for Fe homeostasis and Co/Ni tolerance in rice

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Abstract

Iron (Fe) homeostasis in rice (*Oryza sativa*) requires coordinated uptake and root-to-shoot distribution and can be perturbed by competing transition metals such as cobalt (Co) and nickel (Ni). This study investigated the roles of the metal transporter OsFPN1 in Fe, Co, and Ni transport. OsFPN1–sGFP localized predominantly to the Golgi apparatus. In yeast, OsFPN1 conferred phenotypes consistent with Fe/Co/Ni transport activity. Seedlings carrying CRISPR/Cas9-induced knockout mutations in *OsFPN1* were hypersensitive to iron deficiency (–Fe) and Co/Ni stress; under –Fe supplemented with 100 μ M Ni, seedlings exhibited severe growth inhibition and loss of viability by day 10. Mutant plants accumulated less Fe in shoots but more in roots under –Fe, while Co and Ni treatments resulted in reduced root accumulation and increased shoot translocation. This implies impaired root retention and enhanced long-distance transport of Co and Ni in the mutants. Yeast two-hybrid (Y2H) screening identified 58 non-redundant candidates as OsFPN1-interacting proteins, with interactions of OsPAR1 (a PRA1-family vesicle trafficking factor) and Os12g0168900 (a vacuolar V-ATPase subunit) validated by Y2H and bimolecular fluorescence complementation (BiFC). Reverse transcription quantitative PCR (RT-qPCR) analysis revealed elevated expression of *OsIMA1*, *OsIMA2*, *OsPAR1*, and *Os12g0168900* in mutant shoots, and stronger induction of *OsIMA1*, *OsIMA2*, *OsNAS2*, *OsPAR1*, and *Os12g0168900* in roots specifically under –Fe. These results show that Golgi-localized OsFPN1 is required for proper root-to-shoot Fe allocation and contributes to limiting Co/Ni translocation under metal stress, while the identified protein associations provide candidate links to endomembrane/vacuolar pathways for future functional investigation.

Key Message

Golgi-localized OsFPN1 is essential for root-to-shoot Fe allocation and restricts Co/Ni translocation under metal stress in rice.

Keywords OsFPN1 · Iron deficiency · Cobalt · Nickel · Rice

Introduction

Iron (Fe) is an essential micronutrient that is required by all living organisms. It serves as a cofactor in numerous enzymes and participates in critical processes such as chlorophyll biosynthesis, photosynthesis, respiration, and DNA synthesis (Briat et al. 2015; Connorton and Balk

2019; Kobayashi 2019; Riaz and Guerinot 2021). In plants, Fe deficiency leads to interveinal chlorosis, impaired photosynthesis, stunted growth, and yield loss. Rice (*Oryza sativa*), one of the world's most important staple crops, is particularly sensitive to Fe deficiency during early developmental stages (Chen et al. 2018a, b; Sakariyawo et al. 2020; Mori et al. 1991). In humans, Fe deficiency is the most widespread nutritional disorder, affecting more than 30% of the global population and contributing to anemia, especially among children and pregnant women (Krishna et al. 2023; World Health Organization 2001). Given that rice is a major dietary source of Fe in many regions, understanding its mechanisms of Fe acquisition and homeostasis holds

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both agricultural and nutritional significance (Hanikenne et al. 2021).

Despite its biological importance, Fe availability in soils is often limited due to its predominance as insoluble ferric oxides and hydroxides under aerobic and alkaline conditions (Marschner 1995; Briat et al. 2015). To overcome this, plants have evolved two distinct strategies for Fe uptake. Nongraminaceous species utilize the reduction-based Strategy I, whereas graminaceous plants such as rice employ the chelation-based Strategy II. This involves the biosynthesis and secretion of mugineic acid family phytosiderophores (MAs), which chelate Fe(III) and facilitate its uptake via YSL transporters (Takagi 1976; Curie et al. 2009; Inoue et al. 2009; Nozoye et al. 2011). Rice also possesses the ability to absorb Fe(II) directly through the Fe-regulated transporter OsIRT1 (Ishimaru et al. 2006). These uptake mechanisms, along with nicotianamine (NA)-mediated long-distance transport, are tightly regulated to maintain Fe homeostasis under variable soil conditions (Kobayashi and Nishizawa 2012; Spielmann and Vert 2021). Beyond their role in Fe acquisition, MAs contribute to internal Fe translocation, while their precursor NA supports the mobility of Fe and other divalent metals such as zinc (Zn), copper (Cu), and manganese (Mn) in both graminaceous and nongraminaceous species (Curie et al. 2009; Kobayashi et al. 2019a, b). These findings highlight the interconnected nature of plant metal transport systems and underscore the importance of studying Fe transporters within the broader context of multi-metal homeostasis.

Among transition metals, cobalt (Co) and nickel (Ni) are particularly noteworthy due to their competition with Fe for uptake and transport pathways (Salnikow et al. 2004; Morrissey et al. 2009; Meier et al. 2018). Co is a bioactive trace element with low natural abundance that plays physiological roles in plants (Brengi et al. 2024), such as promoting legume growth (Hu et al. 2021; Gad et al. 2025). However, excessive Co levels can be phytotoxic, disrupting pigment biosynthesis and impairing photosynthetic efficiency (Akeel and Jahan 2020), as observed in tomato (Kamran et al. 2021). At the molecular level, Co serves as a cofactor in enzymes and transcription factors (Banerjee and Bhattacharya 2021; Wang et al. 2020a, b), but high concentrations may lead to nonspecific binding that interferes with essential metal functions (Wang et al. 2018). Ni is an essential micronutrient required at ultratrace levels, functioning as a cofactor for urease and other metalloenzymes, with species-specific roles in certain plants (Amjad et al. 2020; González et al. 2024). Nevertheless, elevated Ni concentrations can negatively affect plant morphology and metabolism (Kováčik et al. 2019; Ul Khair et al. 2020; Naheed et al. 2022). Although generally considered less toxic than other heavy metals (Kováčik et al. 2009 a), Ni has been shown to

be more damaging than cadmium (Cd) at equivalent concentrations in *Taraxacum* (dandelion) (Kováčik et al. 2019).

Plants sense and adapt to fluctuations in heavy metal type and concentration through a coordinated membrane transport network that regulates uptake, long-distance translocation, and subcellular compartmentalization. This network sustains cellular metabolism and mitigates heavy metal stress (Das et al. 2022; Bashir et al. 2021). Within it, ferroportins (FPNs), also known as IREGs, comprise a conserved family of divalent-metal exporters first identified in mammals for their roles in duodenal Fe absorption and macrophage-mediated Fe recycling (McKie et al. 2000; Muckenthaler et al. 2008). Across eukaryotes, FPNs function to extrude Fe from the cytosol, either into the apoplast or into organelles, thereby maintaining intracellular Fe homeostasis (Morrissey et al. 2009; Drakesmith et al. 2015).

Plant IREG/FPNs are classified into two clades (Schaaf et al. 2006; García de la Torre et al. 2021). Group 1, exemplified by *Arabidopsis thaliana* *AtIREG3/MAR1*, encodes plastid-targeted transporters involved in chloroplast and mitochondrial Fe regulation (Kim et al. 2021). Group 2 includes *AtIREG1/FPN1* and *AtIREG2/FPN2*, which localize to the plasma membrane and tonoplast, respectively (Schaaf et al. 2006; Morrissey et al. 2009). *AtFPN1* is expressed in the pericycle plasma membrane and facilitates xylem loading of Co and Fe for shoot delivery, with expression largely independent of Fe status (Morrissey et al. 2009). This process is coupled to FRD3-mediated citrate efflux, which maintains Fe solubility as Fe–citrate complexes (López-Millán et al. 2000; Durrett et al. 2007). In contrast, *AtFPN2* localizes to the vacuolar membrane, is induced under Fe starvation, and sequesters Co and Ni into root vacuoles. Accordingly, *ireg2* mutants exhibit Ni hypersensitivity, whereas *AtIREG2* overexpression enhances Ni tolerance (Schaaf et al. 2006; Morrissey et al. 2009). *AtFPN3/MAR1*, dual-targeted to chloroplasts and mitochondria, functions as an Fe exporter that is essential for organellar Fe balance and also mediates antibiotic entry into chloroplasts (Conte et al. 2010; Conte and Lloyd 2010; Kim et al. 2021).

Comparative studies across species reinforce these functional roles. In the Ni hyperaccumulator *Psychotria gabriellae*, *PgFPN1* is highly expressed, and its transgenic expression in *Arabidopsis* confers increased Ni tolerance (Merlot et al. 2014). In buckwheat (*Fagopyrum esculentum*), *FeFPN1* is root-expressed, strongly induced by Al³⁺ (but not Fe), and enhances Al tolerance when overexpressed in *Arabidopsis*, implying a role in internal Al detoxification (Yokosho et al. 2016). In legumes, the group-2, nodule-specific *Medicago truncatula* Ferroportin2 (*MtFPN2*) encodes an Fe-exporter localized to vascular and symbiosome membranes; its loss-of-function disrupts nodule Fe homeostasis,

reduces nitrogenase activity, and lowers biomass (Escudero et al. 2020).

Although plant FPNs (IREGs) exhibit functional specialization across membranes and tissues, their roles in rice remain poorly characterized. OsFPN1 was previously reported to be a Golgi-localized transporter mediating Co and Ni detoxification (Kan et al. 2022), but unresolved inconsistencies persist. In yeast, *OsFPN1* expression reduced intracellular Co and Ni levels while paradoxically increasing Co hypersensitivity, contrary to expectations that metal efflux should enhance tolerance, as seen with COT1. To clarify these discrepancies, the involvement of OsFPN1 in Fe homeostasis and its role in Co/Ni transport in rice were re-examined in detail.

Materials and methods

Phylogenetic and expression analysis

To investigate the evolutionary relationships of OsFPN1, protein sequences of the FPN/IREG family were retrieved from Phytozome v13, EnsemblPlants, NCBI, and UniProt databases. Representative sequences spanning major plant lineages including monocots, dicots, bryophytes, and algae were selected to ensure broad phylogenetic coverage. Redundant entries were removed prior to analysis. Multiple sequence alignment and phylogenetic tree construction were performed using TBtools v2.127 (Chen et al. 2023). A maximum-likelihood approach was applied to generate the phylogenetic tree, with branch support evaluated through 1,000 bootstrap replicates. Conserved motifs were identified using the MEME suite integrated within TBtools and visualized accordingly.

Tissue-specific expression of *OsFPN1* was assessed using organ and tissue microarray data from the RiceXPro database (Sato et al. 2011, 2013; <http://ricexpro.dna.affrc.go.jp>). The locus *Os06g0560000* (MSU LOC_Os06g36450; ferroportin family) was queried, and probe IDs 25,429 and 30,263 were examined within dataset RXP_0001 (organs and tissues). Cy3 signal intensity values were directly adopted, and bar charts exported from the RiceXPro web interface were presented without further modification (representative plot shown for probe 30263).

To evaluate stress-responsive expression, two published oligonucleotide microarray datasets were reanalyzed. The Fe-deficiency time-course dataset (0–7 days) for the *osiro3* mutant was obtained from Wang et al. (2020a, b), and the multi-metal treatment dataset (+Zn, −Zn, −Fe, ++Cd, −Mn, ++Mn) was obtained from Ogo et al. (2008). Normalized signal intensities were extracted from the original datasets.

For the Fe-deficiency time course, fold-change thresholds of $KO/NT > 2$ (upregulated) and < 0.5 (downregulated) were applied to guide color scaling. For the multi-metal dataset, $\log_2$ -normalized values were used. All expression matrices were row-standardized (Z-score) and visualized as heatmaps using the Heatmap Illustrator module in TBtools v2.127.

Subcellular localization in rice protoplasts

To determine the subcellular localization of OsFPN1 in a homologous system, rice protoplasts were isolated from stems and leaf sheaths of 7- to 15-day-old etiolated seedlings using an enzymatic digestion method. Tissues were cut into fine strips (< 0.5 mm) and digested in an enzyme solution containing 1.5% (w/v) Cellulase R10 (Yakult, Japan), 0.75% (w/v) pectinase (Sangon, China), 0.6 M mannitol, 10 mM CaCl_2 , 10 mM MES (pH 5.7), and 0.1% (w/v) BSA. Digestion was carried out at 28 °C with gentle shaking (20 rpm) for 4–5 h. Released protoplasts were filtered through a 40- μm nylon mesh and collected by centrifugation at 600 rpm for 5 min. The pellet was washed twice with W5 solution (154 mM NaCl, 125 mM CaCl_2 , 2 mM KH_2PO_4 , 2 mM MES, 5 mM glucose, pH 5.7) and resuspended in MMG solution (0.4 M mannitol, 15 mM MgCl_2 , 4 mM MES, pH 5.7) to a final concentration of 2×10^5 cells mL^{-1} .

The OsFPN1–sGFP fusion construct used for rice protoplast transfection was generated by PCR amplification of the *OsFPN1* coding sequence without the stop codon and cloned in-frame with sGFP; the primers used for construct generation are listed in Table S1.

For transfection, 20 μg plasmid DNA (10 μg OsFPN1–sGFP and 10 μg organelle marker construct) was mixed with 200 μL of protoplast suspension and 220 μL of PEG solution (40% PEG4000, 0.2 M mannitol, 100 mM CaCl_2 , pH 5.8). The mixture was incubated at room temperature for 10–15 min, after which the reaction was terminated by adding 1 mL of W5 solution. Protoplasts were collected by centrifugation, washed twice, and resuspended in 1 mL of W5 solution. After incubation at 28 °C in the dark for 18–24 h, fluorescence signals were observed using a confocal laser scanning microscope (Nikon C2-ER, Japan).

For co-localization analysis, OsFPN1–sGFP was co-expressed with organelle markers. Plasma membrane localization was marked using Arabidopsis NAA60 fused with mKate (Linster et al. 2020), and Golgi localization was marked using a Golgi-targeting signal peptide fused with mCherry (Vildanova et al. 2014). Excitation/emission wavelengths were set to 488/510 nm for GFP and 561/580 nm for mKate and mCherry.

Heterologous localization in onion epidermal cells

To examine the subcellular distribution of OsFPN1 in a heterologous expression system, a modified green fluorescent protein (sGFP, S65T) was used as a reporter. The pUC18 vector containing the cauliflower mosaic virus (CaMV) 35 S promoter–sGFP (S65T)–NOS3' cassette was kindly provided by Dr. Yasuo Niwa (University of Shizuoka). The open reading frame of OsFPN1, excluding the stop codon, was amplified by PCR using gene-specific primers introducing *Kpn*I and *Sac*I restriction sites, respectively; the primer sequences are listed in Table S1. The amplified fragment was digested and ligated in-frame into the pUC18-sGFP (S65T)–NOS3' vector. The resulting construct was verified by Sanger sequencing.

Transient transformation of onion (*Allium cepa*) epidermal cells was performed using the Biolistic PDS-1000/He particle delivery system (Bio-Rad, Tokyo, Japan). GFP fluorescence was observed using a confocal laser scanning microscope (LSM5 Pascal, Carl Zeiss, Tokyo, Japan) following the protocol described by Mizuno et al. (2003).

Yeast complementation assays

The full-length coding sequence of *OsFPN1* was cloned into the yeast expression vector pDR195. The recombinant plasmid and the empty vector (VC) were introduced into several *Saccharomyces cerevisiae* mutants, including the Co/Ni-sensitive strain *Δcot1* (MAT α , *trp1-63*, *leu2-3*, *gcn4-101*, *his3-609*, *ura3-52*, *YOR316c/cot1::LEU2*) (Conklin et al. 1992), the Fe uptake-defective double mutant *Δfet3fet4* (MAT α , *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *YMR058w/fet3::kanMX4*; *YMR319c/fet4::kanMX4*) (Ishimaru et al. 2012), the Mn uptake-defective mutant *Δsmf1* (MAT α , *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *YOL122c/smf1::kanMX4*) (Ishimaru et al. 2012), and the Zn uptake-defective mutant *Δzap1/Δzhr6* (Mata, *ade6*, *can1*, *his3*, *leu2*, *trp1*, *ura3*, *YJL056c/zap1::TRP1*) (Zhao and Eide 1997). Yeast transformation was performed using the lithium acetate/PEG method, and two independent colonies were selected for each construct.

For spot assays, yeast cells were precultured overnight in synthetic defined (SD) medium lacking uracil, adjusted to an OD₆₀₀ of 1.0, and serially diluted tenfold (10⁰–10^{−3}). Aliquots (5 μ L) of each dilution were spotted onto SD-uracil-free agar plates supplemented with 1 mM CoCl₂·6H₂O, 1 mM NiCl₂·6H₂O, 50 μ M bathophenanthroline disulfonate (BPDS, –Fe), 50 mM EGTA (–Mn), or 0.1 mM EDTA (–Zn). Control plates without added metals were included. Plates were incubated at 30 °C for 2–3 d, and growth differences between strains harboring *OsFPN1* and the VC were documented.

For growth curve assays, *Δfet3fet4* transformants carrying *OsFPN1* or VC were inoculated into SD-uracil-free liquid medium containing either 0 or 5 μ M BPDS (–Fe), with an initial OD₆₀₀ of 0.05. Cultures were incubated at 30 °C with shaking, and OD₆₀₀ was measured every 10 h up to 40 h to assess growth dynamics.

For intracellular metal accumulation assays, yeast cells expressing *OsFPN1* or VC were cultured in SD-uracil-free liquid medium to an OD₆₀₀ of 0.4, then exposed to 50 μ M CoCl₂·6H₂O or 50 μ M NiCl₂·6H₂O for 1 h at 30 °C. Cells were harvested by centrifugation (8000 $\times$ g, 5 min), washed twice with 50 mL of cold ultrapure water, dried at 70 °C for 48 h, and weighed. Dried pellets were wet-ashed in 2% HNO₃, and intracellular Co and Ni concentrations were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; ISPS-3500, Seiko Instruments Inc., Chiba, Japan).

Yeast two-hybrid (Y2H) screening and validation

A membrane-based split-ubiquitin Y2H system was employed using yeast strain NMY51. The *OsFPN1* bait (*Os06g0560000*) was cloned in-frame into pBT3-STE, and rice cDNA prey library plasmids were prepared using the vector pPR3-N. Prior to large-scale screening, bait toxicity and auto-activation were assessed by co-transforming pBT3-STE-*OsFPN1* with empty pPR3-N onto selective plates: double dropout (DDO, –Leu/–Trp), triple dropout with 3-AT (TDO/3-AT, –Leu/–Trp/–His+3-AT), and quadruple dropout (QDO, –Leu/–Trp/–His/–Ade). Functional competence of the system was verified by co-transforming pOst1-NubI with the bait.

Library transformation was then performed, and primary positives were selected on TDO/X medium. Positive colonies were re-streaked and spotted onto QDO/X to reduce false positives. Prey plasmids from confirmed interactors were rescued, PCR-amplified using CYC1 primers, and sequenced by the Sanger method to identify candidate interactors.

Identified candidates were mapped to rice gene identifiers and subjected to over-representation analysis for Gene Ontology categories (biological process, cellular component, and molecular function) and KEGG pathways in R. P-values were adjusted using the Benjamini–Hochberg method to control the false discovery rate.

For targeted validation, the open reading frames of *OsPAR1* (*Os05g0474400*) and *Os12g0168900* were cloned into pPR3-N and co-transformed with pBT3-STE-*OsFPN1* into NMY51. Transformants were selected on DDO and evaluated on TDO/3-AT and QDO plates. X- α -Gal was included where indicated. Positive (pTSU2-APP/

pNubG-Fe65) and negative (pTSU2-APP/pPR3-N) controls were tested in parallel.

Bimolecular fluorescence complementation (BiFC) assay

To examine protein–protein interactions *in planta*, BiFC assays were performed in *Nicotiana benthamiana* leaves. The full-length coding sequences of *OsFPN1*, *OsPARI* (*Os05g0474400*), and *Os12g0168900* were amplified by PCR using gene-specific primers (listed in Table S1) and cloned into the pSPYCE-35 S and pSPYNE-35 S vectors to generate C-terminal YFP (cYFP) and N-terminal YFP (nYFP) fusion constructs, respectively (Walter et al. 2004). All constructs were verified by sequencing and introduced into *Agrobacterium tumefaciens* strain GV3101 carrying the p19 suppressor.

Agrobacterium cultures were grown in YEB medium, harvested by centrifugation, and resuspended in infiltration buffer containing 10 mM MgCl₂ and 120 μM acetosyringone to an OD₆₀₀ of 0.6. Equal volumes of *Agrobacterium* suspensions carrying *OsFPN1*–cYFP and the respective candidate–nYFP constructs were mixed and infiltrated into the abaxial surface of leaves of 4-week-old *N. benthamiana* plants using a needleless syringe. After infiltration, plants were maintained under low-light conditions for 48 h.

Reconstituted YFP fluorescence was detected using a confocal laser scanning microscope (Olympus FV3000, Japan). Negative controls included co-expression of *OsFPN1*–cYFP with the empty nYFP vector, as well as co-expression of each candidate–nYFP construct with the empty cYFP vector.

CRISPR/Cas9 mutant construction and genotyping

Single-guide RNA (sgRNA) target sites (20 nt upstream of the NGG PAM) within the *OsFPN1* coding sequence were selected using CRISPR-P 2.0 (<http://crispr.hzau.edu.cn/CRISPR2/>) (Liu et al. 2017). Candidate sequences were evaluated for specificity against the rice reference genome. Annealed oligonucleotides were cloned into the pU6gRNA expression cassette (Mikami et al. 2015), which was subsequently inserted into the binary vector pZH_gYSA_MM Cas9 (Endo et al. 2015). The recombinant construct was introduced into *Agrobacterium tumefaciens* strain EHA105 by electroporation.

Agrobacterium-mediated transformation of *Oryza sativa* L. cv. Nipponbare was performed following established protocols (Hiei et al. 1994; Ozawa 2012). Embryogenic calli were infected with *Agrobacterium* harboring the CRISPR/Cas9 construct, co-cultivated on AAM medium in the dark at 25 °C for 3 d, and then washed with sterile

water containing 250 mg/L cefotaxime. Resistant calli were selected on 2N6 medium supplemented with hygromycin B, with stepwise increases in concentrations (10 mg/L for 1 week, 30 mg/L for 1 week, and 50 mg/L for 2 weeks). Hygromycin-resistant calli were transferred to MS regeneration medium under 28 °C and light conditions until shoots developed. Rooted plantlets were acclimatized in soil under greenhouse conditions.

Genomic DNA was extracted from fresh leaves of T1 plants using an NA-2000 automated DNA isolator (Kurabo, Japan). PCR amplification of regions flanking the sgRNA target sites was carried out with gene-specific primers, and purified amplicons were sequenced directly to detect indels. In the T2 generation, plants were further screened to identify homozygous mutants and to select transgene-free individuals segregated from the Cas9 construct.

Plant materials, growth conditions, and hydroponic phenotyping assays

Seeds of *Oryza sativa* L. cv. Nipponbare (wild type) and CRISPR/Cas9-derived mutant lines were used. Dormancy was broken at 37 °C for 5 d, and seeds were surface-sterilized sequentially with 70% (v/v) ethanol for 2 min and 2.5% (w/v) sodium hypochlorite for 30 min with gentle shaking, followed by five rinses with sterile distilled water. Sterilized seeds were germinated on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 50 mg L^{−1} hygromycin B for transformant selection or without hygromycin for wild type. Seedlings were grown at 28 °C under a 14 h light/10-h dark cycle for 2 weeks, acclimatized for 3 d, and then transferred to a hydroponic system.

The nutrient solution contained 0.70 mM K₂SO₄, 0.10 mM KCl, 0.10 mM KH₂PO₄, 2.0 mM Ca(NO₃)₂·4H₂O, 0.50 mM MgSO₄·7H₂O, 10 μM H₃BO₃, 0.50 μM MnSO₄·H₂O, 0.50 μM ZnSO₄·7H₂O, 0.20 μM CuSO₄·5H₂O, and 0.01 μM (NH₄)₆Mo₇O₂₄·4H₂O. For +Fe treatments, 100 μM Fe(III)-EDTA was added (Kobayashi et al. 2019a, b). Seedlings were pre-cultured for 3–5 d until the four-leaf stage and then subjected to the following treatments: (i) +Fe, no additional Co/Ni; (ii) −Fe, no additional Co/Ni; (iii) +Fe + CoCl₂·6H₂O (10 or 100 μM); (iv) +Fe + NiCl₂·6H₂O (10 or 100 μM); (v) −Fe + CoCl₂·6H₂O (10 or 100 μM); and (vi) −Fe + NiCl₂·6H₂O (10 or 100 μM). Nutrient solutions were renewed on day 4 of treatment, and pH was adjusted daily to 5.3–5.5 with 2 M HCl. Plants were cultivated in a greenhouse under natural light at 30 °C (day)/25 °C (night).

For phenotypic evaluation, shoot height and the SPAD values of the youngest fully expanded leaf were measured before treatment (day 0) and after 10 d of treatment using a SPAD-502 chlorophyll meter (Konica Minolta, Tokyo,

Japan). Root length was recorded after 10 d of treatment. For subsequent analyses, leaves and roots from three biological replicates were harvested, either flash-frozen in liquid nitrogen for gene expression analysis or oven-dried for determination of metal concentrations.

ICP-OES analysis of metal concentrations

Shoots and roots were harvested separately, rinsed three times with ultrapure water, and dried at 70 °C for 2–3 d until constant weight was achieved. Approximately 50–100 mg dried tissue was weighed into Teflon digestion vessels and digested with 1.5 mL concentrated HNO₃ (13.4 M) and 1.5 mL H₂O₂ (8.8 M) using a microwave digestion system (MarsXpress, CEM, Matthews, NC, USA) at 220 °C for 20 min. The digested samples were diluted to a final volume of 7 mL with ultrapure water. Elemental concentrations of Fe, Co, Ni, Mn, Zn, and Cu were quantified using ICP-OES (ISPS-3500, Seiko Instruments Inc., Chiba, Japan). Calibration curves were generated using standard solutions of known concentrations, and internal standards were employed to correct for instrumental drift.

RT-qPCR expression analysis

Total RNA was extracted separately from rice shoots and roots using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. First-strand cDNA was synthesized from 1 µg total RNA using ReverTra Ace[®] qPCR RT Master Mix containing gDNA Remover (Toyobo, Osaka, Japan). Quantitative PCR was performed on a LightCycler[®] 480 II Real-Time PCR System (Roche, Basel, Switzerland) using GoTaq[®] qPCR Master Mix (Promega, Madison, WI, USA). Gene-specific primers are listed in Table S1. Transcript levels were normalized to the rice reference gene *OsTUB1* (*tubulin1*), and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Results

Phylogenetic relationships and expression profiles of *OsFPN1*

A maximum-likelihood phylogenetic tree of FPN/IREG proteins from multiple plant species identified four paralogous members in rice (*OsFPN1–4*) (Fig. 1A). *OsFPN1* clustered within the monocot branch, showing the closest relationship to sorghum *Sb10g022120* and clear separation from dicot members such as *AtFPN1/2*. Conserved motif analysis detected 12 motifs across the dataset. All rice members shared similar motif compositions, although

OsFPN4 exhibited a slightly distinct arrangement and length (Fig. 1B).

In the time-course dataset of the knockout mutant of *OsIRO3*, a repressor of Fe homeostasis genes, *OsIMA1/2* (key peptides involved in the Fe deficiency response in rice) were rapidly induced under both Fe-sufficient and early Fe-deficient conditions (days 1–2), followed by a decline after day 3. Comparative clustering identified 21 genes with similar dynamic patterns, including *OsFPN1* in shoot (Fig. S1A). In the metal excess/deficiency treatment dataset, *OsFPN1* was strongly induced under Cd excess and Fe deficiency conditions in shoot, with weaker responses to other treatments (Fig. S1B).

RiceXPro microarray data further revealed spatially heterogeneous expression of *OsFPN1* across tissues and developmental stages (Fig. S2). Strong expression was observed in inflorescences, anthers, pistils, and early seed development, with detectable levels in roots and leaf sheaths. Expression was relatively low in vegetative leaves and late endosperm. Collectively, these findings imply that *OsFPN1* belongs to the rice FPN family and its expression is regulated by Fe availability and developmental stage.

Subcellular localization of *OsFPN1*

To elucidate the subcellular localization of *OsFPN1* in a homologous system, *OsFPN1*–sGFP was transiently expressed in rice protoplasts. Confocal microscopy revealed a punctate intracellular distribution pattern. Co-localization analysis showed that *OsFPN1*–sGFP fluorescence overlapped extensively with the Golgi marker (Fig. 2A), whereas minimal overlap was detected with the plasma membrane marker NAA60 (Fig. 2B). These results indicate that *OsFPN1* preferentially localizes to the Golgi apparatus in rice protoplasts. In contrast, heterologous expression in onion epidermal cells showed peripheral fluorescence enrichment at the plasma membrane (Fig. S3).

OsFPN1 exhibits Co, Ni, and Fe transport activity in yeast cells

Yeast assays were conducted to characterize the transport function of *OsFPN1*. In the Co/Ni-sensitive mutant *Δcot1*, yeast cells expressing *OsFPN1* exhibited reduced growth on SD–uracil-free medium containing 1 mM Co or Ni compared to the vector control (VC), while no difference was observed under control conditions (Fig. 3A). In the Fe-uptake-defective mutant *Δfet3Δfet4*, *OsFPN1* significantly improved colony growth on –Fe medium supplemented with 50 µM BPDS relative to VC (Fig. 3B, left). In contrast, *OsFPN1* did not restore growth in the Mn-deficient mutant *Δsmf1* in the presence of 50 mM EGTA, whereas

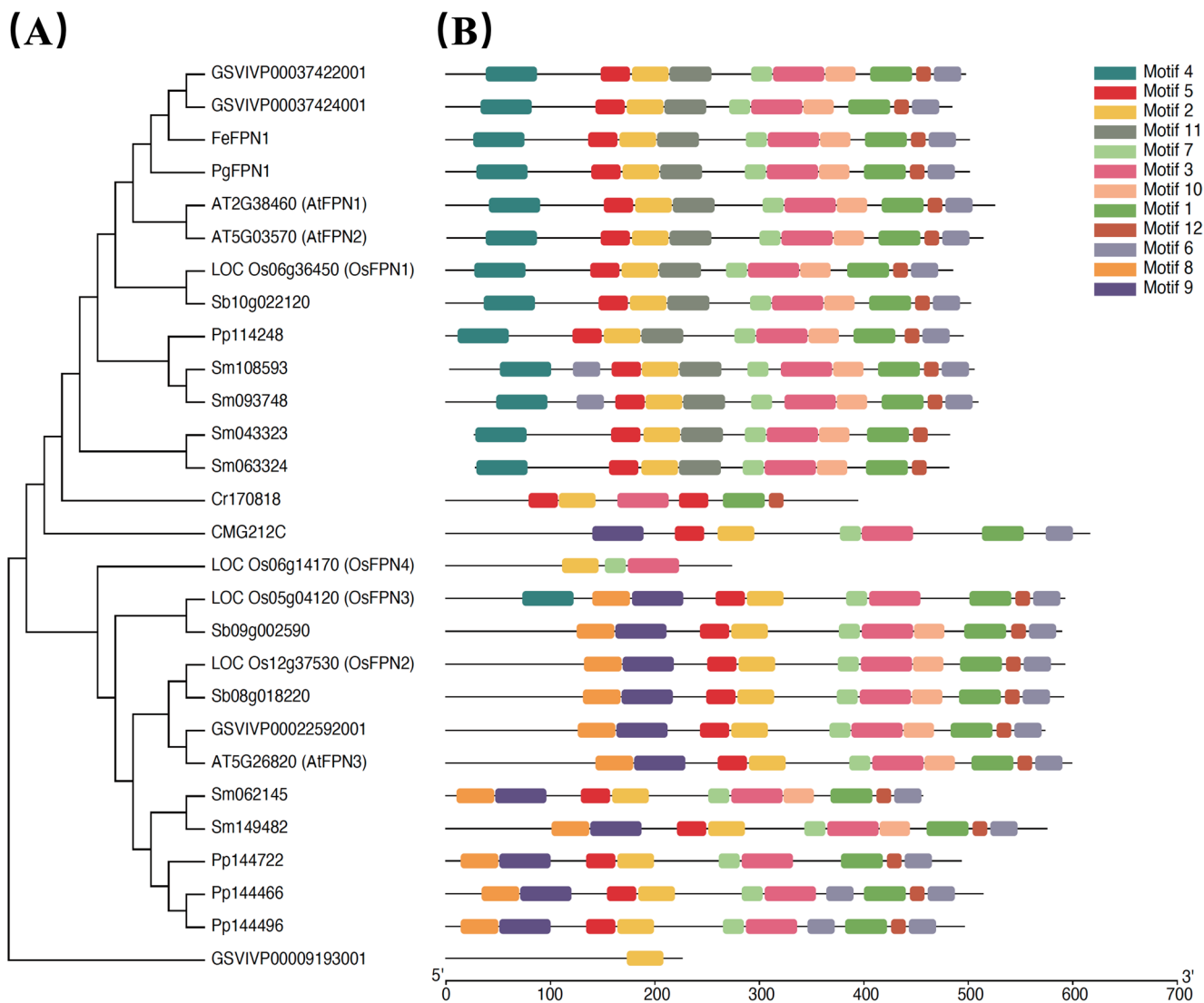


Fig. 1 Phylogenetic relationships and conserved motifs of ferroportin (FPN) family proteins in representative plant species. **A** Phylogenetic tree constructed using the maximum-likelihood method based on full-length amino acid sequences of FPN/IREG family members from *Oryza sativa* (Os), *Sorghum bicolor* (Sb), *Arabidopsis thaliana* (At), *Vitis vinifera* (Vv), *Fagopyrum esculentum* (Fe), *Psychotria gabriel-*

lae (Pg), *Selaginella moellendorffii* (Sm), *Physcomitrella patens* (Pp), *Chlamydomonas reinhardtii* (Cr), and *Cyanidioschyzon merolae* (Cm). The phylogenetic tree was generated using TBtools v2.127 with 1000 bootstrap replicates. **B** Conserved motif analysis performed using the MEME suite integrated in TBtools. Twelve conserved motifs (Motif 1–12) were identified, each represented by a distinct color

the positive control *OsNRAMP5* complemented the defect (Fig. 3B, middle). Similarly, *OsFPN1* failed to complement the Zn-deficient mutant ($\Delta zap1/\Delta zhr6$) under $-Zn$ conditions (Fig. 3B, right). Yeast cells expressing *OsFPN1* accumulated significantly higher levels of Co and Ni than VC (Fig. 3C). In liquid growth assays, *OsFPN1* expression enhanced proliferation of *Afet3Afet4* cells under Fe deficiency, particularly in the presence of 5 μM BPDS (Fig. 3D). These results imply that *OsFPN1* functions as a transporter involved in Fe, Co, and Ni homeostasis.

Y2H screening and validation of *OsFPN1* interactors

To identify protein partners potentially associated with *OsFPN1* function, a split-ubiquitin membrane Y2H screen was performed to identify potential protein partners. Bait quality control confirmed that *OsFPN1* exhibited no auto-activation or toxicity, and system competence was validated using the positive control (Fig. S4). Screening of a rice cDNA library yielded blue colonies on TDO/X, which remained positive on QDO/X (Fig. S5). Colony PCR of rescued prey plasmids produced expected-size inserts (lanes 1–78; Fig. S6). After removing redundancies, 58 non-redundant candidate interactors were retained (Table S2).

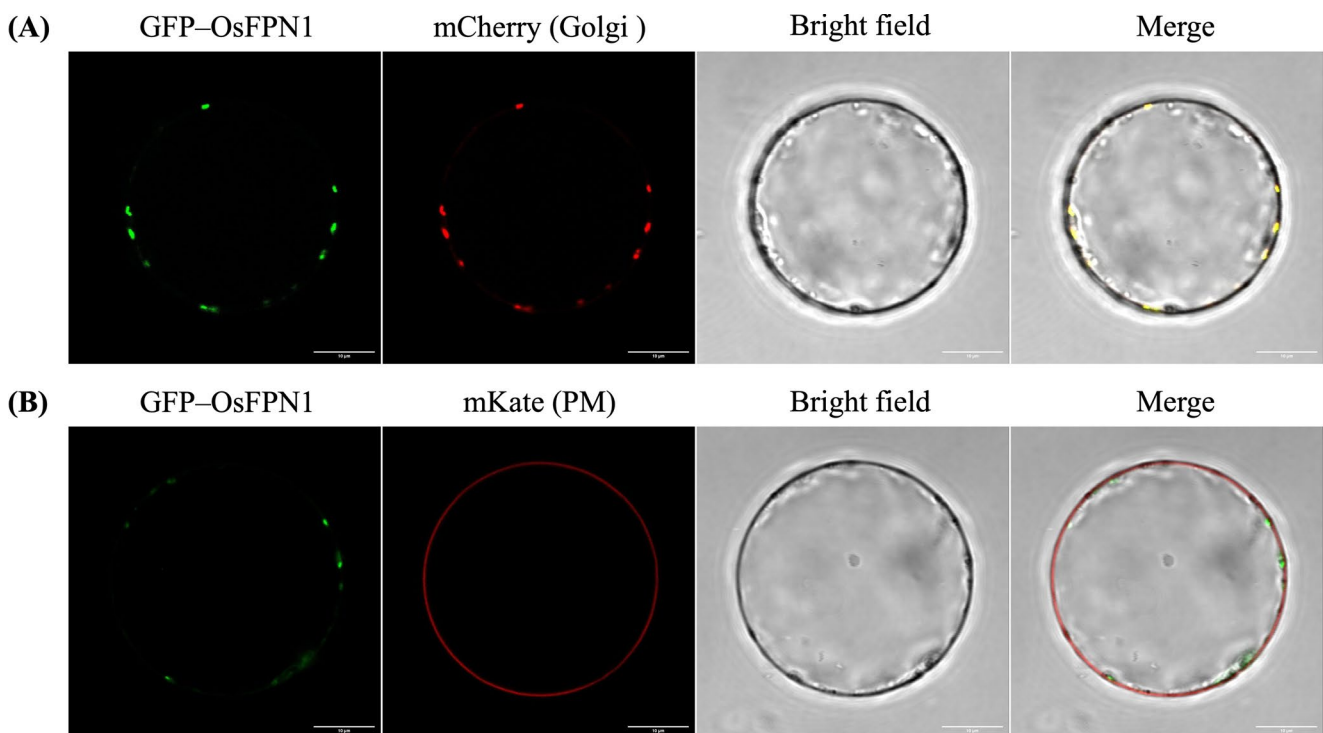


Fig. 2 Subcellular localization of GFP–OsFPN1 in rice protoplasts. **A** Confocal fluorescence images of GFP–OsFPN1 co-expressed with a Golgi marker fused to mCherry in rice protoplasts. **B** Confocal fluorescence images of GFP–OsFPN1 co-expressed with a plasma membrane

(PM) marker fused to mKate in rice protoplasts. From left to right: GFP–OsFPN1 signal (green), organelle marker signal (red), bright-field image, and merged image. Scale bars = 10 μ m

Gene Ontology classification of the 58 selected genes revealed enrichment in biological processes related to cellular activity, localization, and regulation. Cellular component terms were dominated by protein complexes, while molecular function categories were enriched for binding, with additional contributions from transporter and ATP-dependent activity (Fig. S7). KEGG pathway mapping assigned candidates primarily to folding, sorting, and degradation, and translation within Genetic Information Processing, with additional assignments to transport, catabolism, membrane transport, and energy metabolism (Fig. S8).

From these candidates, two proteins were prioritized for validation: OsPAR1 (Os05g0474400), a PRA1-family factor involved in Rab-dependent vacuolar trafficking (Rho et al. 2009; Heo et al. 2010), and Os12g0168900, a V-ATPase subunit required for vacuolar acidification and linked to metal homeostasis (Zhang et al. 2012; Luo et al. 2015; Liang et al. 2021). Serial-dilution assays showed that yeast co-expressing *OsFPN1* with either *OsPAR1* or *Os12g0168900* grew on TDO and QDO, whereas auto-activation and negative controls did not (Fig. 4).

To further assess these interactions in plant cells, BiFC assays were performed in *N. benthamiana* leaves. Reconstituted YFP fluorescence was detected when OsFPN1–cYFP was co-expressed with OsPAR1–nYFP or with

Os12g0168900–nYFP (Figs. S9, S10). These results demonstrate that OsFPN1 forms protein complexes with both OsPAR1 and Os12g0168900 in plant cells, supporting their physical association *in planta*.

These results support physical associations of OsFPN1 with OsPAR1 and Os12g0168900 in heterologous plant cells, and provide candidate leads for exploring whether endomembrane/vacuolar processes contribute to OsFPN1-dependent metal homeostasis in rice.

***osfpn1* mutants are hypersensitive to Fe deficiency and Co/Ni stress**

Two CRISPR/Cas9 mutants, *osfpn1-1* (+1 bp insertion) and *osfpn1-2* (–21 bp deletion), were generated and confirmed (Fig. 5A). Wild type (WT) and *osfpn1* seedlings were compared under hydroponic conditions with Fe sufficiency (+Fe), Fe deficiency (–Fe), and graded Co or Ni supplementation. Under +Fe without Co/Ni, the WT and mutants showed no significant differences in shoot height, SPAD values, or root length (Fig. 5B–D). Under –Fe, all genotypes developed chlorosis, but SPAD decline was significantly greater in *osfpn1* by days 8–10 (Fig. 5B–D). With Co treatment, mutants were hypersensitive. At 10 μ M Co, the *osfpn1* lines exhibited reduced shoot height, aggravated

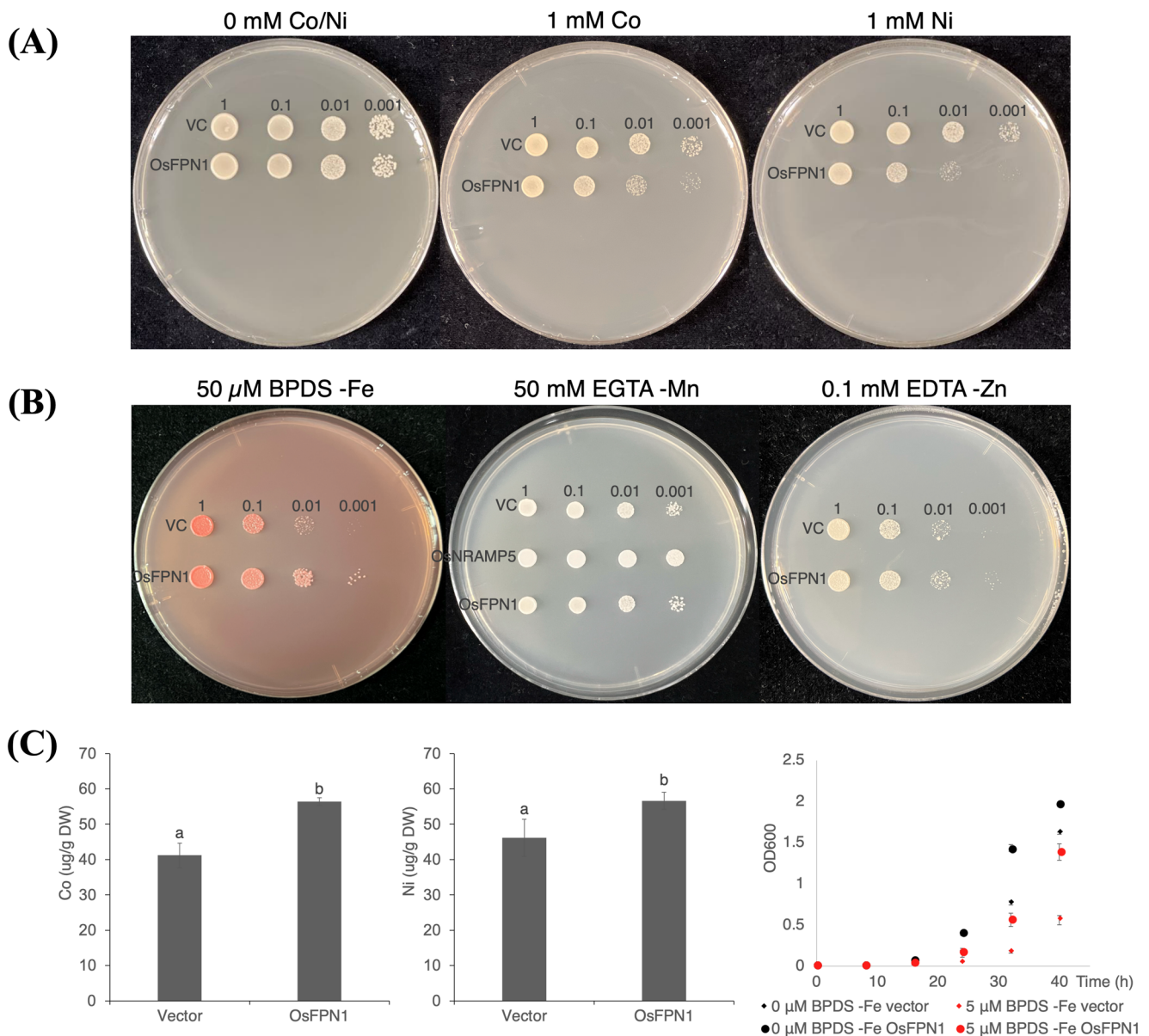


Fig. 3 Functional characterization of OsFPN1 transport activity in yeast. Yeast wild-type and mutant strains were transformed with an empty vector (VC), OsFPN1, or OsNRAMP5, and were analyzed for metal tolerance and transport activity. **A** Spot assays of the Co/Ni-sensitive mutant *Δcot1* carrying VC or OsFPN1 were performed on SD-uracil-free plates containing 0 or 1 mM Co or Ni (10-fold serial dilutions, 10^0 – 10^{-3}). **B** Spot assays of *Δfet3fet4* (–Fe), *Δsmf1* (–Mn), and *Δzap1/Δzhr6* (–Zn) mutants carrying VC, OsFPN1, or OsNRAMP5 were performed on SD-uracil-free plates supplemented

with 50 μ M BPDS, 50 mM EGTA, or 0.1 mM EDTA, respectively. **C** Intracellular Co and Ni contents were measured by ICP-OES ($n=3$) after cells expressing VC or OsFPN1 had been exposed to 50 μ M Co or Ni for 1 h. **D** Growth curves of *Δfet3fet4* transformants harboring VC or OsFPN1 were recorded in –Fe medium containing 0 or 5 μ M BPDS. Data represent the mean $\pm$ SD ($n=3$). Different letters indicate significant differences relative to VC (one-way ANOVA with post hoc Dunnett's test, $p<0.05$). VC, empty vector; OsFPN1, recombinant OsFPN1 vector

chlorosis, and shorter roots compared to the WT (Figs. S11A, S12A, S13A, S15). At 100 μ M Co, inhibition intensified, particularly under –Fe, when mutants showed pronounced chlorosis and strong root suppression (Figs. S11B, S13C, S15). With Ni treatment, mutants displayed more severe phenotypes. At 10 μ M Ni, the *osfpn1* lines showed moderate growth inhibition (Figs. S11C, S12B, S13B, S15).

At 100 μ M Ni, severe chlorosis and root stunting occurred even under +Fe (Figs. S11D, S13D, S14, S15). Under –Fe with 100 μ M Ni, growth declined rapidly after day 5, and mutants were largely non-viable by day 10, whereas the WT remained alive despite growth inhibition (Figs. S11D, S12D, S13D, S14, S15). These results indicate that *OsFPN1* is indispensable for maintaining chlorophyll content and

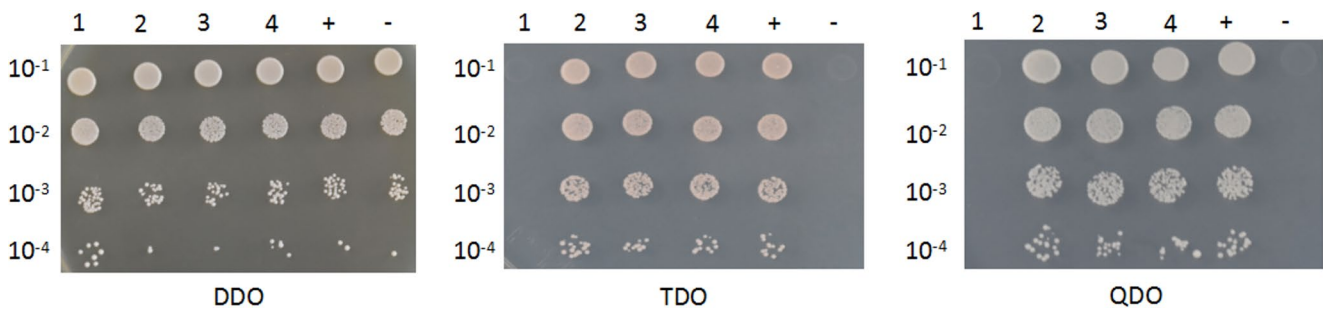


Fig. 4 Y2H point-to-point verification of the interactions between OsFPN1 and OsPAR1/Os12g0168900. Pairwise Y2H assays were carried out in yeast strain *NMY51*. Yeast cells co-transformed with the indicated bait and prey constructs were serially diluted (10^{-1} – 10^{-4}) and spotted onto DDO (–Leu/–Trp), TDO (–Leu/–Trp/–His), and QDO (–Leu/–Trp/–His/–Ade) plates. (1) OsFPN1-pBT3-STE+pPR3-N

(autoactivation control); (2) OsFPN1-pBT3-STE+pOst1-NubI (positive functional control); (3) OsFPN1-pBT3-STE+pPR3-N-OsPAR1 (experimental); (4) OsFPN1-pBT3-STE+pPR3-N-Os12g0168900 (experimental); (+) PTSU2-APP+pNubG-Fe65 (positive interaction control); (–) PTSU2-APP+pPR3-N (negative interaction control)

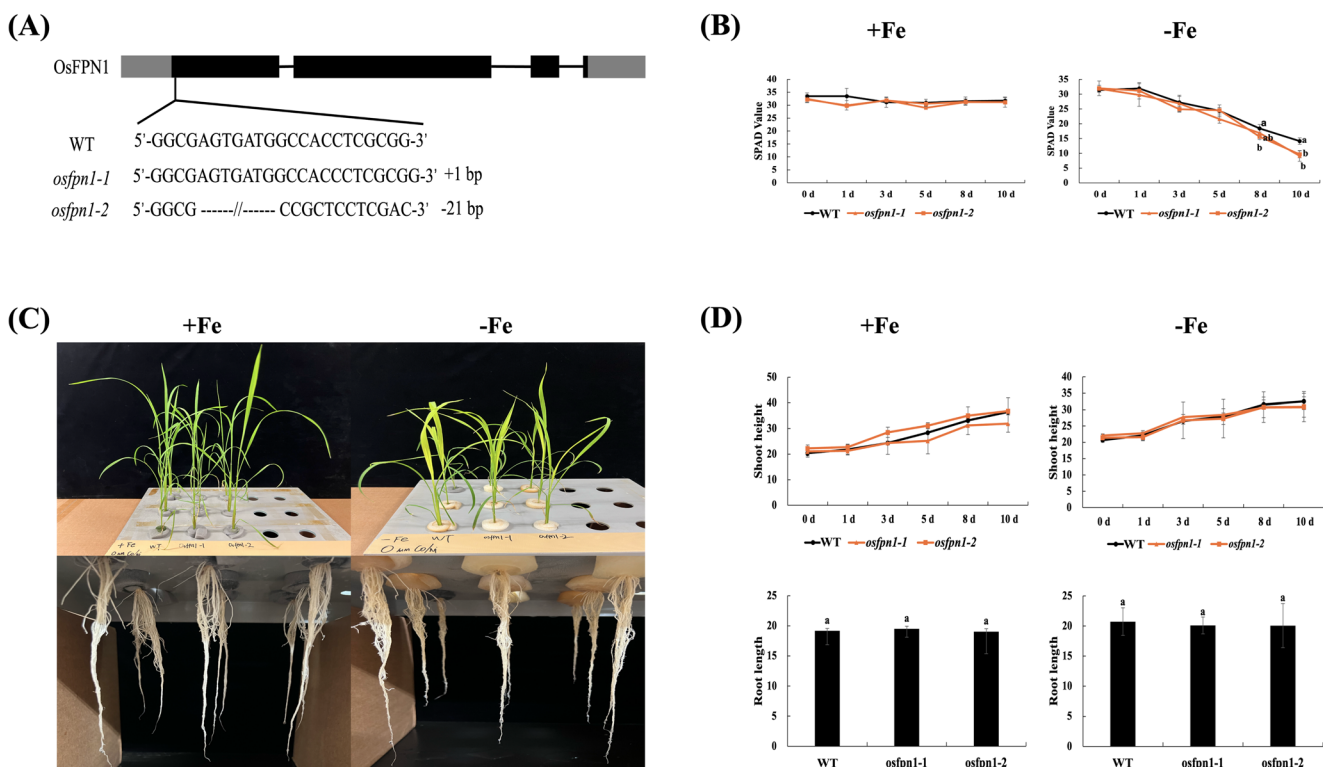


Fig. 5 CRISPR/Cas9-mediated mutagenesis of *osfpn1* and phenotypic analysis of rice plants under different iron conditions. **A** Gene structure of *OsFPN1* showing untranscribed regions (gray boxes), exons (black boxes), and introns (lines). The CRISPR/Cas9 target site and mutation types identified in the mutants (*osfpn1-1*, +1 bp insertion; *osfpn1-2*, –21 bp deletion) are indicated. **B** Time-course analysis of SPAD values in wild type (WT) and *osfpn1* mutants grown under Fe-sufficient (+Fe) and Fe-deficient (–Fe) hydroponic conditions. Data

were recorded at 0, 1, 3, 5, 8, and 10 days. **C** Representative images of WT and mutant plants grown hydroponically under +Fe and –Fe conditions for 10 days. **D** Growth parameters of WT and mutant plants under +Fe and –Fe conditions. Upper panels: shoot height measured at 0, 1, 3, 5, 8, and 10 days. Lower panels: root length measured at day 10. Data represent the mean $\pm$ SD ($n=3$). Different letters indicate significant differences among genotypes (one-way ANOVA followed by Tukey's HSD, $p<0.05$)

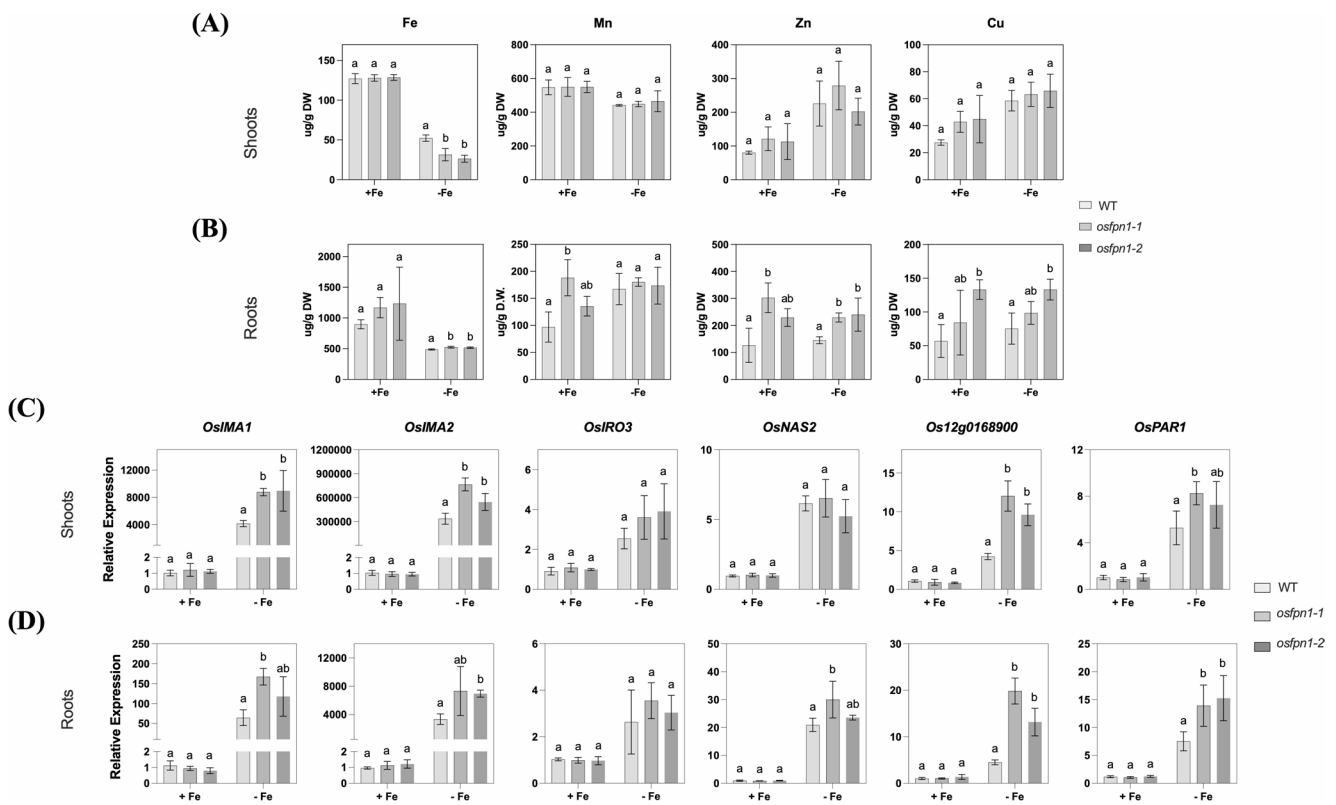


Fig. 6 Metal accumulation and expression analyses in WT and *osfpn1* mutants under Fe-sufficient (+Fe) and Fe-deficient (–Fe) conditions. **A, B** ICP-OES measurements of Fe, Mn, Zn, and Cu concentrations in shoots (**A**) and roots (**B**) of WT, *osfpn1-1*, and *osfpn1-2*. **C, D** RT-qPCR of Fe-deficiency marker genes (*OsIMA1*, *OsIMA2*, *OsIRO3*, and *OsNAS2*) and Y2H candidate interactors (*Os12g0168900* and

OsPAR1) in shoots (**C**) and roots (**D**). Transcript levels were normalized to *OsTUB1* and calculated by the $2^{-\Delta\Delta Ct}$ method. Data represent the mean $\pm$ SD ($n=3$). Different letters indicate significant differences among genotypes (one-way ANOVA followed by Tukey's HSD, $p<0.05$)

root development under Fe limitation, and plays a critical role in protecting rice seedlings against Co and Ni toxicity.

osfpn1 mutants show altered metal accumulation depending on Fe status and Co/Ni exposure

To evaluate the role of *OsFPN1* in metal homeostasis, concentrations of Fe, Mn, Zn, Cu, Co, and Ni were measured in shoots and roots of the WT and the *osfpn1* mutants under +Fe and –Fe conditions, with or without Co/Ni supplementation. In the absence of Co or Ni supplementation, mutant shoots accumulated significantly less Fe under –Fe compared to the WT, while Zn and Cu tended to increase under +Fe (Fig. 6A). The roots of mutants exhibited elevated Fe, Mn, Zn, and Cu under both +Fe and –Fe conditions (Fig. 6B). Under Co treatment, distinct changes were observed. At 10 μ M Co, mutant shoots showed reduced Fe and elevated Co, along with modest increases in Zn and Cu (Fig. S16A), whereas roots accumulated more Mn, Zn, and Cu but less Co (Fig. S16B). At 100 μ M Co, shoots again contained lower Fe and higher Co, especially under –Fe (Fig. S16C), while roots accumulated more Fe, Zn, and Cu

but less Co (Fig. S16D). Under Ni treatment, similar alterations were evident. At 10 μ M Ni, mutant shoots exhibited significantly reduced Fe and increased Ni (Fig. S16E), while roots accumulated more Zn and Cu but less Ni (Fig. S16F). At 100 μ M Ni, shoots showed marked Fe reduction and Ni elevation under both +Fe and –Fe conditions (Fig. S16G), whereas roots displayed strong Fe enrichment but reduced Ni (Fig. S16H). These findings demonstrate that disruption of *OsFPN1* perturbs Fe partitioning between shoots and roots, alters Mn/Zn/Cu balance, and impairs Co/Ni sequestration in roots, thereby enhancing their relative accumulation in shoots under stress conditions.

OsFPN1 interacts with *OsPAR1* and *Os12g0168900* and modulates Fe-deficiency signaling

To assess whether *OsFPN1* disruption influences the expression of Fe-deficiency marker genes and its identified interactors, qRT-PCR was conducted in shoots and roots of the WT and *osfpn1* under +Fe and –Fe conditions. Under +Fe, no significant differences were detected between the WT and mutants. Under –Fe, mutant shoots showed

significantly elevated expression of *OsIMA1*, *OsIMA2*, *OsPAR1*, and *Os12g0168900* compared to the WT (Fig. 6C). In roots, mutants displayed enhanced induction of *OsIMA1*, *OsIMA2*, *OsNAS2*, *OsPAR1*, and *Os12g0168900* under $-Fe$ (Fig. 6D). These results indicate that loss of *OsFPN1* leads to enhanced induction of Fe-deficiency-responsive genes in both shoots and roots, including those encoding the protein interactors OsPAR1 and Os12g0168900, under Fe-deficient conditions.

Discussion

Ferroportin/IREG proteins constitute an evolutionarily conserved family of divalent-metal transporters that are broadly present across eukaryotic lineages. In animals, FPNs mediate duodenal Fe absorption and macrophage Fe recycling, thereby maintaining systemic Fe homeostasis (McKie et al. 2000; Muckenthaler et al. 2008). Homologous transporters have also been identified in plants, where they share conserved transmembrane structures and metal-binding motifs with their animal counterparts but have diversified in subcellular localization, substrate preference, and physiological function (Schaaf et al. 2006; Morrissey et al. 2009; García de la Torre et al. 2021). In this study, phylogenetic analysis identified four rice paralogs (*OsFPN1-4*), consistent with previous reconstructions of the soybean IREG family that included the same four rice sequences (Cai et al. 2020). The conservation of the FPN/IREG family across eukaryotes, together with its diversification within plant lineages, indicates that these transporters retain a common evolutionary framework for transition-metal regulation, while exhibiting species-specific adaptations in localization and physiological function.

The functional role of OsFPN1 has remained unclear. Kan et al. (2022) reported predominant Golgi localization in rice protoplasts and proposed a role in Co/Ni transport, while plasma membrane signals were also detected in yeast. In this study, OsFPN1-sGFP exhibited a predominantly punctate pattern in rice protoplasts and showed extensive co-localization with a Golgi marker (Fig. 2A), whereas overlap with the plasma membrane marker NAA60 was limited and only occasionally observed (Fig. 2B). In a heterologous system, transient expression in onion epidermal cells resulted in peripheral fluorescence enrichment at the plasma membrane (Fig. S3). These observations suggest that OsFPN1 is primarily associated with the Golgi in a homologous rice system, while a minor plasma membrane pool can be detected depending on cellular context and expression system, potentially reflecting trafficking through the secretory pathway or altered sorting under heterologous expression conditions. Dynamic relocalization between

the plasma membrane and internal membranes has been reported for other plant metal transporters. In *Arabidopsis*, the Fe^{2+} transporter IRT1 cycles between the plasma membrane and endosomal/vacuolar compartments via monoubiquitin-dependent endocytosis (Barberon et al. 2011). IRT1 also functions as a metal sensor, detecting cytosolic Zn^{2+} and Mn^{2+} via a histidine-rich loop, which triggers CIPK23-dependent phosphorylation and IDf1-mediated ubiquitination, leading to internalization and vacuolar targeting (Dubeaux et al. 2018; Spielmann et al. 2023). Similarly, the Mn^{2+} transporter NRAMP1 undergoes phosphorylation-dependent, clathrin-mediated endocytosis upon Mn resupply, cycling between the plasma membrane and endosomes to prevent toxicity (Castaings et al. 2021). These findings indicate that regulated trafficking is a common post-translational mechanism for metal transporters and provide a biological context to interpret the varying localization patterns observed for OsFPN1 across different expression systems.

A possible link between OsFPN1 and endomembrane/vacuolar processes is suggested by protein–protein interaction assays. Y2H assays supported interactions of OsFPN1 with OsPAR1, a PRA1-family factor involved in Rab-dependent trafficking (Rho et al. 2009; Heo et al. 2010), and with Os12g0168900, a V-ATPase subunit required for vacuolar acidification and linked to metal-related processes (Zhang et al. 2012; Luo et al. 2015; Liang et al. 2021) (Fig. 4). Importantly, these associations were further supported in plant cells by BiFC assays in *N. benthamiana*, where reconstituted YFP fluorescence was detected for OsFPN1 with OsPAR1 and with Os12g0168900 (Figs. S9, S10). These interaction data are consistent with the involvement of OsFPN1 in vesicle- and vacuole-related pathways and are compatible with the possibility that endomembrane-associated processes contribute to intracellular metal distribution.

Multiple independent lines of evidence imply that *OsFPN1* contributes to Fe homeostasis. Transcriptome reanalysis in *osiro3* mutants placed *OsFPN1* in the same dynamic cluster as the early Fe-deficiency markers *OsIMA1* and *OsIMA2*, showing rapid induction at days 1–2 of Fe starvation followed by decline (Fig. S1A) (Wang et al. 2020a, b). Previous datasets likewise showed strong *OsFPN1* induction under Fe deficiency and Cd excess (Fig. S1B) (Ogo et al. 2008). Functionally, *osfpn1* mutants retained more Fe in roots but showed reduced Fe accumulation in shoots under Fe deficiency (Fig. 6A, B). Consistent with enhanced Fe-deficiency signaling, mutants exhibited elevated transcript levels in shoots for *OsIMA1*, *OsIMA2*, *OsPAR1*, and *Os12g0168900* (Fig. 6C), and in roots for *OsIMA1*, *OsIMA2*, *OsNAS2*, *OsPAR1*, and *Os12g0168900* (Fig. 6D), while *OsIRO3* expression remained unchanged. No significant differences were detected under Fe-sufficient conditions. These results indicate that *OsFPN1* facilitates

Fe partitioning between roots and shoots, implying that metal loading into the endomembrane system may contribute to efficient root-to-shoot translocation. Although the precise trafficking route linking Golgi-localized OsFPN1 to xylem loading remains unresolved, the central role of the Golgi in secretory trafficking provides a plausible cellular framework through which intracellular metal partitioning can influence long-distance transport. Loss of OsFPN1 thus enhances Fe-deficiency-responsive transcription, contrasting with previous conclusions that OsFPN1 is dispensable for Fe nutrition (Kan et al. 2022). Earlier reviews have also reported Fe-deficiency-responsive changes in *OsFPN1* expression (Ricachenevsky et al. 2018), supporting the view that *OsFPN1* transcriptionally responds to Fe status.

The results further demonstrate that *OsFPN1* is essential for coping with Co and Ni exposure by supporting root sequestration and retention, thereby limiting long-distance translocation. Even under +Fe, Co or Ni supplementation caused strong chlorosis in *osfpn1* seedlings (Figs. S11, S12). ICP-OES profiling revealed consistent redistribution under Co/Ni treatment: mutants accumulated less Co/Ni in roots but more in shoots compared to the WT (Fig. S16), reflecting impaired root sequestration and enhanced shoot loading. This suggests that OsFPN1 may normally sequesters excess Co/Ni into the endomembrane system to limit their entry into the transpiration stream. Such patterns are consistent with competition between Co/Ni and Fe for transport pathways (Salnikow et al. 2004; Morrissey et al. 2009; Meier et al. 2018). In mung bean, Co toxicity is largely attributable to inhibition of Fe translocation to shoots (Liu et al. 2000), consistent with the Co-induced Fe enrichment in rice roots observed here (Fig. S16A–D). Overlapping specificities among transporters (for instance, OsMTP1 handles Fe/Co/Ni) likely intensify competitive interactions (Menguer et al. 2013; Shirazi et al. 2019; Gao et al. 2020). Because Co stress often produces Fe-deficiency-like symptoms (Roychoudhury and Chakraborty 2022), additive effects of Co exposure and Fe deficiency in *osfpn1* are expected.

Ni produced particularly severe effects. Under –Fe plus 100 μ M Ni, *osfpn1* seedlings collapsed after day 5 and were largely non-viable by day 10 (Figs. S11D, S12D, S13D). Although Ni is an essential micronutrient required only in trace amounts (Amjad et al. 2020; González et al. 2024), its toxicity increases rapidly above the physiological range and can even exceed that of Cd in some species (Kováčik et al. 2019). The observed lethality under combined –Fe and high Ni highlight the vulnerability of rice metabolism to Ni overload when Fe delivery to shoots is impaired.

In summary, OsFPN1 functions as a regulator of Fe partitioning between roots and shoots in rice. Loss of *OsFPN1* caused Fe accumulation in roots, reduced Fe levels in shoots, and enhanced activation of Fe-deficiency signaling

pathways. In addition, *osfpn1* mutants showed increased sensitivity to Co and Ni stress, indicating impaired buffering capacity and stronger competition between Fe and these metals. While preliminary protein–protein interaction data hint at potential links to vesicle and vacuolar trafficking, these results primarily refine the functional model of OsFPN1 as an essential metal transporter and identify it as a potential molecular target for improving Fe use efficiency and enhancing tolerance to Co and Ni stress in crops.

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Author contributions JDW and HN designed the research. JDW performed most of the experiments and wrote the manuscript. HN supervised the research and oversaw project administration. Both authors contributed to funding acquisition.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interests The authors declare no competing interests.

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References

- Akeel A, Jahan A (2020) Role of Cobalt in Plants: Its Stress and Alleviation. In: Naeem M, Ansari AA, Gill SS (eds) Contaminants in agriculture: sources, impacts and management, pp 339–357
- Amjad M, Ameen N, Murtaza B, Imram M, Shahid M, Abbas G, Naeem NA, Jacobsen SE (2020) Comparative physiological and biochemical evaluation of salt and nickel tolerance mechanisms in two contrasting tomato genotypes. *Physiol Plant* 168:27–37

- Banerjee P, Bhattacharya P (2021) Investigating cobalt in soil–plant–animal–human system: dynamics, impact and management. *J Soil Sci Plant Nutr* 21:2339–2354
- Barberon M, Zelazny E, Robert S, Conéjéro G, Curie C, Friml J, Vert G (2011) Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) controls iron uptake in plants. *Proc Natl Acad Sci USA* 108:E450–E458
- Bashir K, Ahmad Z, Kobayashi T, Seki M, Nishizawa NK (2021) Roles of subcellular metal homeostasis in crop improvement. *J Exp Bot* 72:2083–2098
- Brengi SH, Moubarak M, El-Naggar HM, Osman AR (2024) Promoting salt tolerance, growth, and phytochemical responses in coriander (*Coriandrum sativum* L cv Balady) via eco-friendly *Bacillus subtilis* and cobalt. *BMC Plant Biol* 24:848
- Briat JF, Dubos C, Gaymard F (2015) Iron nutrition, biomass production, and plant product quality. *Trends Plant Sci* 20:33–40
- Cai Z, Xian P, Lin R, Cheng Y, Lian T, Ma Q, Nian H (2020) Characterization of the soybean GmIREG family genes and the function of GmIREG3 in conferring tolerance to aluminum stress. *Int J Mol Sci* 21:497
- Castangs L, Caquot A, Loubet S, Curie C (2021) The NRAMP1 metal transporter contributes to Mn homeostasis in *Arabidopsis* via dynamic changes in subcellular localization. *Plant J* 106:1561–1576
- Chen L, Wang G, Chen P, Zhu H, Wang S, Ding Y (2018a) Shoot-root communication plays a key role in physiological alterations of rice (*Oryza sativa*) under iron deficiency. *Front Plant Sci* 9:757
- Chen PF, Chen L, Jiang ZR, Wang GP, Wang SH, Ding YF (2018b) Sucrose is involved in the regulation of iron deficiency responses in rice (*Oryza sativa* L). *Plant Cell Rep* 37:789–798
- Chen C, Wu Y, Li J, Wang X, Zeng Z, Liu Y, Feng J, Chen H, He Y, Xia R (2023) TBtools-II: a one for all, all for one bioinformatics platform for biological big-data mining. *Mol Plant* 16:1733–1740
- Conklin DS, McMaster JA, Culbertson MR, Kung C (1992) COT1, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. *Mol Cell Biol* 12:3678–3688
- Connorton JM, Balk J (2019) Iron biofortification of staple crops: lessons and challenges in plant genetics. *Plant Cell Physiol* 60:1447–1456
- Conte SS, Lloyd AM (2010) The MAR1 transporter is an opportunistic entry point for antibiotics. *Plant Signal Behav* 5(1):49–52
- Conte S, Stevenson D, Furner I, Lloyd A (2010) Multiple antibiotic resistance in *Arabidopsis* is conferred by mutations in a chloroplast-localized transport protein. *Plant Physiol* 151:559–573
- Curie C, Cassin G, Couch D, Divol F, Higuchi K, Le Jean M, Misson J, Schikora A, Czerniec P, Mari S (2009) Metal movement within the plant: contribution of nicotianamine and yellow stripe 1-like transporters. *Ann Bot* 103:1–11
- Das N, Tirunagari P, Maiti MK (2022) Plant metal tolerance proteins: insight into their roles in metal transport and homeostasis for future biotechnological applications. In: Kumar K, Srivastava S (eds) *Plant Metal and Metalloid Transporters*. Springer Nature Singapore, Singapore, pp 289–304
- de la García VS, Majorel-Loulergue C, Rigai GJ, Alfonso-González D, Soubigou-Taconnat L, Pillon Y, Barreau L, Thomine S, Fogliani B, Burtet-Sarramegna V, Merlot S (2021) Wide cross-species RNA-seq comparison reveals convergent molecular mechanisms involved in nickel hyperaccumulation across dicotyledons. *New Phytol* 229:994–1006
- Drakesmith H, Nemeth E, Ganz T (2015) Ironing out Ferroportin. *Cell Metab* 22:777–787
- Dubeaux G, Neveu J, Zelazny E, Vert G (2018) Metal sensing by the IRT1 transporter–receptor orchestrates its own degradation and plant metal nutrition. *Mol Cell* 69:953–964e5
- Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. *Plant Physiol* 144:197–205
- Endo M, Mikami M, Toki S (2015) Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice. *Plant Cell Physiol* 56:41–47
- Escudero V, Abreu I, Tejada-Jiménez M, Rosa-Núñez E, Quintana J, Prieto RI, Larue C, Wen J, Villanova J, Mysore KS, Argüello JM, Castillo-Michel H, Imperial J, González-Guerrero M (2020) *Medicago truncatula* Ferroportin2 mediates iron import into nodule symbiosomes. *New Phytol* 228:194–209
- Gad N, Elrahman EAA, Ali MEF, Abou-Shlell MK, Teiba II, Almutairi MH, Yousef AF (2025) Role and importance of cobalt in faba bean through rationalization of its nitrogen fertilization. *BMC Plant Biol* 25:872
- Gao Y, Yang F, Liu J, Xie W, Zhang L, Chen Z, Peng Z, Ou Y, Yao Y (2020) Genome-wide identification of metal tolerance protein genes in *Populus trichocarpa* and their roles in response to various heavy metal stresses. *Int J Mol Sci* 21:1680
- González DA, de la Torre VSG, Fernández RR, Barreau L, Merlot S (2024) Divergent roles of IREG/Ferroportin transporters from the nickel hyperaccumulator *Leucocroton havanensis*. *Physiol Plant* 176:e14261
- Hanikenne M, Esteves SM, Fanara S, Rouached H (2021) Coordinated homeostasis of essential mineral nutrients: a focus on iron. *J Exp Bot* 72:2136–2153
- Heo JB, Bang WY, Kim SW, Hwang SM, Im YS, Son CH, Acharya BR, Kim CW, Kim SW, Lee BH, Bahk JD (2010) OsPRA1 plays a significant role in targeting of OsRab7 into the tonoplast via the prevacuolar compartment during vacuolar trafficking in plant cells. *Planta* 232:861–871
- Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant J* 6:271–282
- Hu X, Wei X, Ling J, Chen J (2021) Cobalt: an essential micronutrient for plant growth? *Front Plant Sci* 12:768523
- Inoue H, Kobayashi T, Nozoye T, Yamakawa T, Takahashi M, Mori S, Nishizawa NK (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
- Ishimaru Y, Suzuki M, Tsukamoto T, Nakanishi H, Matsushashi S, Takahashi M, Mori S, Nishizawa NK (2006) Rice plants take up iron as an Fe³⁺-phytosiderophore and as Fe²⁺. *Plant J* 45:335–346
- Ishimaru Y, Takahashi R, Bashir K, Shimo H, Senoura T, Sugimoto K, Ono K, Yano M, Ishikawa S, Arao T, Nakanishi H, Nishizawa NK (2012) Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Nat Commun* 3:1050
- Kamran M, Danish M, Saleem MH, Imram M, Shahid M, Abbas G, Naeem NA, Jacobsen SE (2021) Application of abscisic acid and 6-benzylaminopurine modulated morpho-physiological and antioxidant defense responses of tomato (*Solanum lycopersicum* L.) by minimizing cobalt uptake. *Chemosphere* 263:128169
- Kan M, Fujiwara T, Kamiya T (2022) Golgi-localized OsFPN1 is involved in Co and Ni transport and their detoxification in rice. *Rice* 15:36
- Kim LJ, Tsuyuki KM, Hu F, Park EY, Zhang J, Iraheta JG, Chia J, Huang R, Tucker AE, Clyne M, Castellano C, Kim A, Chung DD, DaVeiga CT, Parsons EM, Vatamaniuk OK, Jeong J (2021) Ferroportin 3 is a dual-targeted mitochondrial/chloroplast iron exporter necessary for iron homeostasis in *Arabidopsis*. *Plant J* 107:215–236
- Kobayashi T (2019) Understanding the complexity of iron sensing and signaling cascades in plants. *Plant Cell Physiol* 60:1440–1446

- Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. *Annu Rev Plant Biol* 63:131–152
- Kobayashi T, Nozoye T, Nishizawa NK (2019a) Iron transport and its regulation in plants. *Free Radical Biol Med* 133:11–20
- Kobayashi T, Ozu A, Kobayashi S, An G, Jeon JS, Nishizawa NK (2019b) OsbHLH058 and OsbHLH059 transcription factors positively regulate iron deficiency responses in rice. *Plant Mol Biol* 101:471–486
- Kováčik J, Gruz J, Hedbavny J, Klejdus B, Strand M (2009) Cadmium and nickel uptake are differentially modulated by salicylic acid in *Matricaria chamomilla* plants. *J Agric Food Chem* 57:9848–9855
- Kováčik J, Bujdoš M, Ketzer P, Babula P, Peterková V, Krenn L (2019) Dandelion is more tolerant to cadmium than to nickel excess. *Chemosphere* 224:884–891
- Krishna TPA, Ceasar SA, Mahajan T (2023) Biofortification of crops to fight anemia: role of vacuolar iron transporters. *J Agric Food Chem* 71:3583–3598
- Liang G, Song H, Xiao Y, Zhang Z (2021) Ammonium accumulation caused by reduced tonoplast V-ATPase activity in *Arabidopsis thaliana*. *Int J Mol Sci* 22:12
- Linster E, Loyer D, Bienvenut WV, Wirtz M, Mengel P, Cohen GH, Noudoghe A, Meinel T, Giglione C, Hell R, Wanke D (2020) The *Arabidopsis* Na-acetyltransferase NAA60 locates to the plasma membrane and is vital for the high salt stress response. *New Phytol* 228:554–569
- Liu J, Reid RJ, Smith FA (2000) The mechanism of cobalt toxicity in mung beans. *Physiol Plant* 110:104–110
- Liu H, Ding Y, Zhou Y, Jin W, Xie K, Chen LL (2017) CRISPR-P 2.0: an improved CRISPR-Cas9 tool for genome editing in plants. *Mol Plant* 10:530–532
- López-Millán AF, Morales FN, Abadía J, Abadía J (2000) Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet: implications for iron and carbon transport. *Plant Physiol* 124:873–884
- Luo Y, Scholl S, Doering A, Zhang Y, Irani NG, Di Rubbo S, Neumetzler L, Krishnamoorthy P, Van Houtte I, Mylle E, Bischoff V, Vernhettes S, Winne J, Friml J, Stierhof YD, Schumacher K, Persson S, Russinova E (2015) V-ATPase activity in the TGN/EE is required for exocytosis and recycling in *Arabidopsis*. *Nat Plants* 1:15094
- Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
- McKie AT, Marciari P, Rolfs A, Brennan K, Wehr K, Barrow D, Miret S, Bomford A, Peters TJ, Farzaneh F, Hediger MA, Hentze MW, Simpson RJ (2000) A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. *Mol Cell* 5:299–309
- Meier SK, Adams N, Wolf M, Balkwill K, Muasya AM, Gehring CA, Bishop JM, Ingle RA (2018) Comparative RNA-seq analysis of nickel hyperaccumulating and non-accumulating populations of *Senecio coronatus* (Asteraceae). *Plant J* 95:1023–1038
- Menguer PK, Farthing E, Peaston KA, Ricachenevsky FK, Fett JP, Williams LE (2013) Functional analysis of the rice vacuolar zinc transporter OsMTP1. *J Exp Bot* 64:2871–2883
- Merlot S, Hannibal L, Martins S, Martinelli L, Amir H, Lebrun M, Thomine S (2014) The metal transporter PglREG1 from the hyperaccumulator *Psychotria gabriellae* is a candidate gene for nickel tolerance and accumulation. *J Exp Bot* 65:1551–1564
- Mikami M, Toki S, Endo M (2015) Comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. *Plant Mol Biol* 88:561–572
- Mizuno D, Higuchi K, Sakamoto T, Nakanishi H, Mori S, Nishizawa NK (2003) Three nicotianamine synthase genes isolated from maize are differentially regulated by iron nutritional status. *Plant Physiol* 132:1989–1997
- Mori S, Nishizawa N, Hayashi H, Chino M, Yoshimura E, Ishihara J (1991) Why are young rice plants highly susceptible to iron deficiency? In: Chen Y, Hadar Y (eds) Iron nutrition and interactions in plants. Proceedings of the Fifth International Symposium on Iron Nutrition and Interactions in Plants, Jerusalem, June 1989. Developments in Plant and Soil Sciences, vol 43. Springer, Dordrecht, p 175–188
- Morrissey J, Baxter IR, Lee J, Li L, Lahner B, Grotz N, Kaplan J, Salt DE, Gueriot ML (2009) The ferroportin metal efflux proteins function in iron and cobalt homeostasis in *Arabidopsis*. *Plant Cell* 21:3326–3338
- Muckenthaler MU, Galy B, Hentze MW (2008) Systemic iron homeostasis and the iron-responsive element/iron-regulatory protein (IRE/IRP) regulatory network. *Annu Rev Nutr* 28:197–213
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473–497
- Naheed N, Abbas G, Naeem MA, Hussain M, Shabbir R, Alamri S, Siddiqui MH, Mumtaz MZ (2022) Nickel tolerance and phytoremediation potential of quinoa are modulated under salinity: multivariate comparison of physiological and biochemical attributes. *Environ Geochem Health* 44:1409–1424
- Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Mori S, Nishizawa NK (2011) Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *J Biol Chem* 286:5446–5454
- Ogo Y, Kobayashi T, Itai RN, Nakanishi H, Kakei Y, Takahashi M, Toki S, Mori S, Nishizawa NK (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
- Ozawa K (2012) A high-efficiency *Agrobacterium*-mediated transformation system of rice (*Oryza sativa* L.). *Methods Mol Biol* 847:51–57
- Rho HS, Heo JB, Bang WY, Hwang SM, Nahm MY, Kwon HJ, Kim SW, Lee BH, Bahk JD (2009) The role of OsPRA1 in vacuolar trafficking by OsRab GTPases in plant system. *Plant Sci* 177:411–417
- Riaz N, Gueriot ML (2021) All together now: regulation of the iron deficiency response. *J Exp Bot* 72:2045–2055
- Ricachenevsky FK, de Araújo Junior AT, Fett JP, Sperotto RA (2018) You shall not pass: root vacuoles as a symplastic checkpoint for metal translocation to shoots and possible application to grain nutritional quality. *Front Plant Sci* 9:412
- Roychoudhury A, Chakraborty S (2022) Cobalt and molybdenum: deficiency, toxicity, and nutritional role in plant growth and development. In: Naeem M, Ansari AA, Gill SS (eds) Plant Nutrition and Food Security in the Era of Climate Change. Academic, Cambridge, pp 255–270
- Sakariyawo OS, Oyediji OE, Soretire AA (2020) Effect of iron deficiency on the growth, development and grain yield of some selected upland rice genotypes in the rainforest. *J Plant Nutr* 43:851–863
- Salnikow K, Donald SP, Bruick RK, Zhitkovich A, Phang JM, Kasprzak KS (2004) Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. *J Biol Chem* 279:40337–40344
- Sato Y, Antonio BA, Namiki N, Takehisa H, Minami H, Kamatsuki K, Sugimoto K, Shimizu Y, Hirochika H, Nagamura Y (2011) RiceXPro: a platform for monitoring gene expression in *japonica* rice grown under natural field conditions. *Nucleic Acids Res* 39:D1141–D1148
- Sato Y, Takehisa H, Kamatsuki K, Minami H, Namiki N, Ikawa H, Ohyanagi H, Sugimoto K, Antonio BA, Nagamura Y (2013) RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Res* 41:D1206–D1213

- Schaaf G, Honsbein A, Meda AR, Kirchner S, Wipf D, von Wirén N (2006) AtIREG2 encodes a tonoplast transport protein involved in iron-dependent nickel detoxification in *Arabidopsis thaliana* roots. *J Biol Chem* 281:25532–25540
- Shirazi Z, Abedi A, Kordrostami M, Burritt DJ, Hossain MA (2019) Genome-wide identification and characterization of the metal tolerance protein (MTP) family in grape (*Vitis vinifera* L.). *3 Biotech* 9:199
- Spielmann J, Vert G (2021) The many facets of protein ubiquitination and degradation in plant root iron-deficiency responses. *J Exp Bot* 72:2071–2082
- Spielmann J, Fanara S, Cotellet V, Vert G (2023) Multilayered regulation of iron homeostasis in Arabidopsis. *Front Plant Sci* 14:1250588
- Takagi S (1976) Naturally occurring iron-chelating compounds in oat- and rice-root washing. I. Activity measurement and preliminary characterization. *Physiol Plant Nutr* 22:423–433
- Ul Khair K, Farid M, Ashraf U, Zubair M, Rizwan M, Farid S, Ishaq HK, Iftikhar U, Ali S (2020) Citric acid enhanced phytoextraction of nickel (Ni) and alleviate *Mentha piperita* (L) from Ni-induced physiological and biochemical damages. *Environ Sci Pollut Res* 27:27010–27022
- Vildanova MS, Wang W, Smirnova EA (2014) Specific organization of Golgi apparatus in plant cells. *Biochem (Mosc)* 79:894–906
- Walter M, Chaban C, Schütze K, Batistič O, Weckermann K, Näke C, Blazevic D, Grefen C, Schumacher K, Oecking C, Harter K, Kudla J (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J* 40:428–438
- Wang FH, Qiao K, Liang S, Tian SQ, Wang H, Chai TY (2018) *Triticum urartu* MTP1: its ability to maintain Zn²⁺ and Co²⁺ homeostasis and metal selectivity determinants. *Plant Cell Rep* 37:1653–1666
- Wang F, Itai RN, Nozoye T, Kobayashi T, Nishizawa NK, Nakanishi H (2020a) The bHLH protein OsIRO3 is critical for plant survival and iron (Fe) homeostasis in rice (*Oryza sativa* L.) under Fe-deficient conditions. *Soil Sci Plant Nutr* 66:579–592
- Wang YM, Yang Q, Xu H, Liu YJ, Yang HL (2020b) Physiological and transcriptomic analysis provide novel insight into cobalt stress responses in willow. *Sci Rep* 10:2308
- World Health Organization (2001) Iron deficiency anemia assessment prevention and control: a guide for program managers. World Health Organization, Geneva
- Yokosho K, Yamaji N, Mitani-Ueno N, Shen RF, Ma JF (2016) An aluminum-inducible IREG gene is required for internal detoxification of aluminum in buckwheat. *Plant Cell Physiol* 57:1169–1178
- Zhang Y, Xu YH, Yi HY, Gong JM (2012) Vacuolar membrane transporters OsVIT1 and OsVIT2 modulate iron translocation between flag leaves and seeds in rice. *Plant J* 72:400–410
- Zhao H, Eide DJ (1997) Zap1p, a metalloregulatory protein involved in zinc-responsive transcriptional regulation in *Saccharomyces cerevisiae*. *Mol Cell Biol* 17:5044–5052

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