OsIMA1 Enhances Rice Adaptation to Cadmium Stress Postprint

Authors: Peng Feng, Lu Chengkai, Liang Gang

Date: 2022-06-29T00:00:00+00:00

Abstract

Iron (Fe) is an essential nutrient element for plant growth and development, whereas cadmium (Cd) is a detrimental element to plants, and antagonistic interactions exist in the absorption of Fe and Cd by plants. OsIMA represents a class of small peptides that positively regulate Fe uptake in rice, and their overexpression can promote Fe accumulation. To investigate whether OsIMA participates in rice adaptation to Cd stress, this study employed rice as the experimental material, analyzed the expression levels of OsIMA genes via quantitative real-time PCR, constructed OsIMA1-overexpressing plants and ima1 mutant plants through genetic transformation and CRISPR/Cas9 gene editing technology, evaluated the plant height of OsIMA1-overexpressing and mutant plants under Cd stress conditions, and measured Fe and Cd contents in roots and shoots using inductively coupled plasma mass spectrometry. The results demonstrated that: (1) Following Cd treatment, the transcription levels of OsIMA1 and OsIMA2 were upregulated; (2) OsIMA1-overexpressing plants exhibited enhanced tolerance to Cd stress compared with wild-type plants; (3) ima1 loss-of-function mutants displayed increased sensitivity to Cd stress relative to wild-type plants; (4) Cd content analysis revealed that Cd accumulation was elevated in the roots of OsIMA1-overexpressing plants, whereas Cd accumulation was higher in the shoots of ima1 mutant plants. In conclusion, OsIMA1 enhances rice adaptation to Cd adversity by restricting Cd transport from roots to shoots, thereby providing a theoretical foundation for the directional breeding of Cd-tolerant crops.

Full Text

OsIMA1 Enhances Rice Tolerance to Cadmium Stress

Peng Feng[12], **Lu Chengkai**[12], **Liang Gang**[12]* [1]CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming 650223, China [2]College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: Iron (Fe) is an essential nutrient for plant growth and development, whereas cadmium (Cd) is a toxic element. Plant uptake of Fe and Cd exhibits antagonistic interactions. OsIMAs are a class of small peptides that positively regulate Fe uptake in rice, and their overexpression promotes Fe accumulation. To investigate whether OsIMA participates in rice adaptation to Cd stress, we analyzed OsIMA gene expression levels using quantitative real-time PCR, constructed OsIMA1-overexpressing and ima1 mutant plants via genetic transformation and CRISPR/Cas9 gene editing, evaluated plant height under Cd stress conditions, and measured Fe and Cd contents in roots and shoots using inductively coupled plasma mass spectrometry. The results showed that: (1) Cd treatment upregulated the transcription levels of both OsIMA1 and OsIMA2; (2) OsIMA1-overexpressing plants exhibited greater tolerance to Cd stress than wild-type plants; (3) ima1 loss-of-function mutants were more sensitive to Cd stress than wild-type plants; and (4) Cd content analysis revealed that OsIMA1-overexpressing plants accumulated higher Cd levels in roots, whereas ima1 mutants accumulated more Cd in shoots. In summary, OsIMA1 enhances rice adaptation to Cd stress by restricting Cd translocation from roots to shoots, providing a theoretical basis for the targeted breeding of Cd-tolerant crops.

Keywords: rice, OsIMA, cadmium, iron, antagonism

Introduction

Cadmium (Cd) is a toxic heavy metal element harmful to all living organisms that readily competes with other divalent metal ions (such as Fe and Zn) during root uptake from soil. Cd can accumulate in the human body through the food chain, and long-term accumulation can lead to osteoporosis, cancer, and kidney dysfunction. With rapid industrialization and excessive use of fertilizers and pesticides, increasing Cd contamination in soils has seriously threatened food security, posing potential risks to human health and causing substantial economic losses (Kirkham, 2006; Wei & Yang, 2010). Therefore, investigating the molecular mechanisms of Cd uptake in plants and screening or breeding crops with Cd stress tolerance or low Cd accumulation benefits both human health and ecological protection.

Due to their similar hydrated ionic radii, Cd can readily enter plants by competing with Fe through Fe ion channels or transporters (Nightingale, 1959; Eide et al., 1996; Schutzendubel & Polle, 2002; Clemens, 2006). Cd accumulation in plants causes obvious chlorosis and necrosis in young leaves, growth inhibition, and even death—symptoms similar to typical Fe deficiency (Das et al., 1997; Cohen et al., 1998; Yoshihara et al., 2006). Studies have shown that Cd stress alters cell wall composition, enhances cell wall binding capacity for Fe, and results in Fe retention in root apoplasts while inhibiting Fe translocation from roots to shoots (Xu et al., 2015). These symptoms can be alleviated by Fe supplementation (Bao et al., 2010; He et al., 2017; Huang et al., 2020). Research indicates that the Fe transporter IRT1 (Iron-Regulated Transporter 1) participates in Cd uptake (Cohen et al., 1998; Korshunova et al., 1999; Vert et al., 2002; Fan et al., 2014; Mao et al., 2014; Guan et al., 2019; Zhu et al., 2020). In Arabidopsis, IRT1 expression is directly regulated by the transcription factor FIT (FER-like Iron deficiency-induced Transcription factor) and members of the bHLH Ib subfamily (bHLH38, bHLH39, bHLH100, and bHLH101) (Yuan et al., 2008). Co-expression of FIT with bHLH38 or bHLH39 enhances plant tolerance to Cd stress by increasing Cd sequestration in roots and reducing Cd accumulation in shoots (Wu et al., 2012). The transcription factor bHLH104 positively regulates Fe homeostasis in Arabidopsis, and its overexpression improves plant tolerance to Cd (Zhang et al., 2015; Li et al., 2016; Yao et al., 2018). BTS (BRUTUS) negatively regulates Fe homeostasis, and BTS knockout significantly enhances Arabidopsis tolerance to Cd stress while increasing Fe and Cd accumulation (Kobayashi et al., 2013; Hindt et al., 2017; Zhu et al., 2020). In rice, two Fe transporters, OsNRAMP1 (Natural Resistance-Associated Macrophage Protein 1) and OsIRT1, can mediate Cd uptake from soil, and overexpression of OsNRAMP1 and OsIRT1 increases rice Cd content (Lee & An, 2009; Takahashi et al., 2019). Due to the antagonistic relationship between Fe and Cd, Fe deficiency in rice significantly increases root Cd uptake, while exogenous Fe application reduces rice Cd content and alleviates Cd toxicity (Shao et al., 2007). In summary, improving Fe accumulation and distribution can significantly mitigate Cd toxicity in plants. Therefore, a comprehensive understanding of Fe-Cd antagonism can guide agricultural production to reduce Cd toxicity.

Small peptides, generally defined as proteins with fewer than 100 amino acids, contain short conserved amino acid motifs and participate in plant development and responses to abiotic stresses (Czyzewicz et al., 2013; Matsubayashi, 2014). IMA (IRON MAN) family peptides contain a 17-amino-acid conserved motif at their C-terminus that is highly conserved in angiosperms, and these 17 amino acids alone are sufficient to function in place of the full-length protein (Grillet et al., 2018; Li et al., 2021). Recently, small peptides have received widespread attention as hormone-like signaling molecules involved in organ communication, plant development, and stress responses (Takahashi et al., 2019; Tavormina et al., 2015). IMA may mediate long-distance signaling of Fe from shoots to roots, participating in Fe uptake, translocation, and intracellular homeostasis (Grillet et al., 2018; Li et al., 2021). Recent studies have shown that IMA overexpression can improve Cd stress tolerance in Arabidopsis (Meng et al., 2022). However, whether IMA can enhance Cd stress tolerance in crops remains unclear. This study investigated the role of rice OsIMA1 in regulating Cd stress to provide a theoretical basis for future molecular breeding to improve rice tolerance to Cd stress.

1. Materials and Methods

1.1 Plant Materials

The experimental material was the japonica rice variety "Nipponbare,"cultivated at the Crop Protection and Breeding Base of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, located in Menglun Town, Mengla County, Xishuangbanna Dai Autonomous Prefecture, Yunnan Province (101°19 E, 21°52 N). Rice seeds were soaked in double-distilled water (ddH_2O) for 24 h, then evenly spread on absorbent filter paper and placed in darkness at 28°C for 3 days. Germinated seeds were transferred to 1/2 MS nutrient solution for 7 days, then moved to 1/2 MS hydroponic solution without Cd or containing 25 M cadmium chloride ($CdCl_2$) for an additional 7 days. The hydroponic solution was replaced every 2 days. The growth light cycle was 16 h light/8 h dark.

1.2 Generation of Transgenic Plants

CRISPR-GE (http://skl.scau.edu.cn/) was used to design editing targets for OsIMA1. Overlapping PCR was employed to construct sgRNA containing the target sequence, which was fused downstream of the OsU6a promoter. The OsU6a-sgRNA cassette was then cloned into the pMH-SA vector carrying Cas9 (Liang et al., 2016). The expression vector was transformed into *Agrobacterium tumefaciens* strain EHA105 for rice transformation, and transgenic positive seedlings were identified by PCR sequencing. Homozygous plants were propagated, and T_3 generation plants were further verified by PCR sequencing to obtain homozygous mutant lines.

For OsIMA1 overexpression, the full-length CDS of OsIMA1 was driven by the maize ubiquitin promoter. The constructed plasmid was transformed into *Agrobacterium tumefaciens* strain EHA105 for rice transformation. Transgenic plants with hygromycin resistance were selected for gene expression analysis, and T_3 generation transgenic plants were used for experimental analysis.

1.3 Quantitative Real-Time PCR (qRT-PCR)

Total RNA from rice roots or shoots was extracted using the water-saturated phenol method. RNA was reverse-transcribed into cDNA using RT Primer Mix (oligo dT) and PrimeScript RT Enzyme Mix for qPCR (Takara, Japan). qRT-PCR was subsequently performed using the PrimeScript™ RT reagent (Perfect Real Time) Kit (Takara, Japan) on a LightCycler 480 real-time PCR system (Roche, Switzerland). Each gene quantification included at least three biological replicates. OsACTIN1 and OsOBP were used as internal reference controls for sample normalization.

1.4 Fe and Cd Content Determination

Different rice genotypes were germinated in 1/2 MS nutrient solution for 7 days, then transferred to 1/2 MS hydroponic solution without Cd or containing 25

M $CdCl_2$ for 7 days. Roots and leaves were harvested separately and dried in a 65°C oven for 7 days. Samples were ground into powder using a high-throughput tissue grinder. For each sample, 500 mg of powder was digested with 5 mL nitric acid at 185°C for 3 h, followed by oxidation with 2 mL perchloric acid at 220°C for 30 min. Elemental contents were determined using an inductively coupled plasma mass spectrometer (ICP-MS iCAP6300, Thermo SCIENTIFIC, USA).

1.5 Statistical Analysis

All experimental data are presented as mean ± standard deviation (SD). Each experiment included at least three biological replicates. One-way analysis of variance (ANOVA) was performed using SPSS 17.0 software ($P < 0.05$).

2. Results and Analysis

2.1 Cd Stress Induces Upregulation of OsIMA Genes in Rice

The rice genome contains two OsIMA genes (OsIMA1 and OsIMA2). Recent studies have shown that OsIMA1 and OsIMA2 are induced by Fe deficiency. Cd stress severely limits plant growth and development, causing plants to exhibit Fe deficiency symptoms. To investigate whether Cd stress could affect OsIMA1 and OsIMA2 expression, we examined their expression patterns in wild-type rice after Cd treatment. Seedlings grown in normal nutrient solution for 7 days were transferred to solution without Cd or containing 25 M $CdCl_2$ for 7 days. RNA was extracted from roots and shoots, and expression levels of OsIMA1 and OsIMA2 were detected by qRT-PCR. The results showed that under Cd stress conditions, the expression of both OsIMA1 and OsIMA2 was significantly upregulated in both roots and shoots [Figure 1: see original paper].

2.2 OsIMA1-Overexpressing Plants Exhibit Higher Cd Tolerance

OsIMA1 and OsIMA2 are homologous proteins with similar functions in Fe homeostasis. Considering that the transcription levels of both genes were induced by Cd treatment, this study focused on OsIMA1 as a representative. We used the maize Ubiquitin promoter to drive OsIMA1 expression and transformed the wild-type rice variety "Nipponbare" [Figure 2: see original paper]A. OsIMA1-overexpressing transgenic rice lines were screened by quantitative PCR. We then evaluated the Cd stress adaptation of these overexpression plants. Seedlings grown in normal hydroponic solution for one week were transferred to solution without Cd (Cd0) or containing 25 M $CdCl_2$ (Cd25) for another week, after which phenotypes were observed and analyzed. The results showed that under Cd0 conditions, OsIMA1-overexpressing plants showed no obvious difference from wild-type plants. However, under Cd25 treatment, overexpression plants were significantly taller than wild-type plants [Figure 2: see original paper]B and C. These results indicate that OsIMA1 overexpression enhances plant adaptation to Cd stress.

2.3 OsIMA1 Loss-of-Function Mutants Are More Sensitive to Cd Stress

To further investigate the role of OsIMA1 in Cd stress adaptation, we used CRISPR-Cas9 gene editing technology to edit the OsIMA1 gene and obtained two ima1 mutant lines. We designed a specific target in the OsIMA1 coding region, used the OsU6a promoter to drive sgRNA expression, and constructed the corresponding expression vector. Transgenic plants were obtained by transforming wild-type rice callus. Through PCR identification and subsequent segregation, two homozygous mutant lines, ima1-1 and ima1-2, were obtained. Both lines contained a single-base insertion in the coding region, causing frameshift mutations [Figure 3: see original paper]A. We then performed Cd treatment experiments on wild-type and the two loss-of-function mutants. The results showed that under normal hydroponic conditions, both mutant lines showed no obvious difference from wild-type plants [Figure 3: see original paper]B and C. Under Cd treatment, however, mutant plants exhibited symptoms such as chlorosis and even wilting of young leaves [Figure 3: see original paper]B. These results demonstrate that loss of OsIMA1 function increases plant sensitivity to Cd.

2.4 OsIMA1 Negatively Regulates Cd Translocation from Roots to Shoots

Our results showed that OsIMA1-overexpressing plants were more tolerant to Cd stress than wild-type, whereas ima1 mutants showed the opposite phenotype. To further analyze the molecular mechanism by which OsIMA1 positively regulates Cd tolerance, we determined Cd and Fe contents in roots and leaves of wild-type, OsIMA1-overexpressing, and ima1 mutant plants. Seedlings grown in normal nutrient solution for 7 days were transferred to solution without Cd or containing 25 M $CdCl_2$ for 7 days, after which roots and leaves were harvested to measure Fe and Cd contents. Fe concentration analysis showed that compared with wild-type, OsIMA1-overexpressing plants accumulated more Fe in both roots and shoots, whereas ima1 mutants accumulated less Fe [Figure 4: see original paper]. Cd concentration analysis revealed that OsIMA1-overexpressing plants accumulated more Cd in roots, while ima1 mutants accumulated more Cd in shoots [Figure 4: see original paper]. These results indicate that OsIMA1 negatively regulates Cd transport from roots to shoots.

Discussion

Cd is detrimental to the growth and development of animals and plants, and rice contamination by Cd has occurred frequently in recent years. Therefore, reducing Cd uptake in plants or improving plant adaptation to Cd stress benefits food security. Fe is an essential nutrient for plant growth and development, and Fe deficiency is a major cause of anemia in humans. As a staple food crop, rice is a primary source of dietary Fe for humans, making Fe-enriched rice beneficial for human health. Due to the antagonistic relationship between Fe and Cd, Fe supplementation can reduce plant Cd uptake (Wu et al., 2012; Sebastian & Prasad, 2016). Thus, exploiting the Fe-Cd antagonistic mechanism can improve plant adaptation to Cd stress. Rice contains two OsIMA genes, OsIMA1 and OsIMA2, whose overexpression activates Fe deficiency-induced genes and increases Fe accumulation (Kobayashi et al., 2021). This study found that Cd stress significantly upregulated the expression of both OsIMA1 and OsIMA2 [Figure 1: see original paper]. Cd treatment experiments revealed that loss-of-function mutants of OsIMA1 increased rice sensitivity to Cd, primarily manifested as leaf chlorosis and wilting [Figure 3: see original paper]. OsIMA1 overexpression not only increased Fe accumulation but also inhibited Cd translocation from roots to shoots [Figure 4: see original paper], which may account for the enhanced Cd tolerance in overexpression plants. Therefore, increasing OsIMA1 expression levels represents an effective strategy for alleviating Cd toxicity in plants.

A recent study demonstrated that IMA overexpression improves Cd stress adaptation in Arabidopsis (Meng et al., 2022). This study found that OsIMA1 overexpression enhances Cd stress tolerance in rice [Figure 3: see original paper]. Fe and Cd taken up by rice roots are transported to shoots and ultimately to grains. OsIMA1 overexpression promotes Fe accumulation in roots while simultaneously promoting Cd accumulation in roots. However, while OsIMA1 overexpression enhances Fe accumulation in shoots, it inhibits Cd translocation from roots to shoots. This suggests that OsIMA1 can specifically inhibit Cd transport from roots to shoots, thereby improving plant adaptation to Cd stress. Both Fe and Cd are divalent ions that may be recognized by similar transporters in vivo. The Fe accumulated in roots of OsIMA1-overexpressing plants may competitively inhibit Cd binding to these transporters. The ability of OsIMA1 to restrict Cd translocation from roots to shoots may help reduce Cd accumulation in rice grains. Future studies should test whether OsIMA overexpression can reduce Cd content in rice grains. The fact that IMA increases Fe uptake and enhances Cd tolerance in both Arabidopsis and rice suggests that IMA function is conserved across species. The high conservation of IMA may help overcome species-specific limitations of gene function (Grillet et al., 2018). Therefore, future research should test whether IMA can improve Cd tolerance in other crops.

Studies have shown that the 17 amino acids at the C-terminus of the IMA peptide are sufficient for its molecular function (Grillet et al., 2018; Li et al., 2021). Currently, artificial synthesis of small peptide technology has become well-established. Therefore, IMA could potentially be developed as a commercial peptide to improve plant Cd stress tolerance through fertilization. In summary, IMA represents a potential small molecule for alleviating Cd toxicity in plants. Further investigation of the mechanisms by which IMA regulates Cd stress will provide a theoretical basis for genetically engineering plants or breeding Cd-tolerant crops.

References

Bao T, Sun LN, Sun TH, 2010. Evaluation of iron on cadmium uptake by tomato, morel and leaf red beet in hydroponic culture[J]. J Plant Nutr, 33(5): 713-723.

Clemens S, 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants[J]. Biochimie, 88(11): 1707-1719.

Cohen CK, Fox TC, Garvin DF, et al., 1998. The role of iron-deficiency stress responses in stimulating heavy-metal transport in plants[J]. Plant Physiol, 116(3): 1063-1072.

Czyzewicz N, Yue K, Beeckman T, et al., 2013. Message in a bottle: small signalling peptide outputs during growth and development[J]. J Exp Bot, 64(17): 5281-5296.

Das P, Samantaray S, Rout GR, 1997. Studies on cadmium toxicity in plants: a review[J]. Environ Pollut, 98(1): 29-36.

Eide D, Broderius M, Fett J, et al., 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast[J]. Proc Natl Acad Sci USA, 93(11): 5624-5628.

Fan SK, Fang XZ, Guan MY, et al., 2014. Exogenous abscisic acid application decreases cadmium accumulation in Arabidopsis plants, which is associated with the inhibition of IRT1-mediated cadmium uptake[J]. Front Plant Sci, 5(1): 721.

Grillet L, Lan P, Li W, et al., 2018. IRON MAN is a ubiquitous family of peptides that control iron transport in plants[J]. Nat Plants, 4(11): 953-963.

Guan MY, Zhu YX, Liu XX, et al., 2019. Induction of S-nitrosoglutathione reductase reduces root cadmium uptake by inhibiting Iron-regulated transporter 1[J]. Plant Soil, 438(1): 315-325.

He XL, Fan SK, Zhu J, et al., 2017. Iron supply prevents Cd uptake in Arabidopsis by inhibiting IRT1 expression and favoring competition between Fe and Cd uptake[J]. Plant Soil, 416(1): 165-176.

Hindt MN, Akmakjian GZ, Pivarski KL, et al., 2017. BRUTUS and its paralogs, BTS LIKE1 and BTS LIKE2, encode important negative regulators of the iron deficiency response in Arabidopsis thaliana[J]. Metallomics, 9(7): 876-890.

Huang G, Ding C, Li Y, et al., 2020. Selenium enhances iron plaque formation by elevating the radial oxygen loss of roots to reduce cadmium accumulation in rice (Oryza sativa L.) [J]. J Hazard Mat, 398(1): 122860.

Kirkham MB, 2006. Cadmium in plants on polluted soils: effects of factors, hyperaccumulation, and amendments[J]. Geoderma, 137(1): 19-32.

Kobayashi T, Nagano AJ, Nishizawa NK, 2021. Iron deficiency-inducible peptide coding genes OsIMA1 and OsIMA2 positively regulate a major pathway of iron uptake and translocation in rice[J]. J Exp Bot, 72(6): 2196-2211.

Kobayashi T, Nagasaka S, Senoura T, et al., 2013. Iron-binding haemerythrin RING ubiquitin ligases regulate plant iron responses and accumulation[J]. Nat Commun, 4(1): 2792.

Korshunova YO, Eide D, Clark WG, et al., 1999. The IRT1 protein from Arabidopsis thaliana is a metal transporter with a broad substrate range[J]. Plant Mol Biol, 40(1): 37-44.

Lee S, An G, 2009. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice[J]. Plant Cell Environ, 32(4): 408-416.

Li J, Liu JC, Yan CL, et al., 2019. The alleviation effect of iron on cadmium phytotoxicity in mangrove A. marina. Alleviation effect of iron on cadmium phytotoxicity in mangrove Avicennia marina (Forsk.) Vierh[J]. Chemosphere, 226(1): 413-420.

Li X, Zhang H, Ai Q, et al., 2016. Two bHLH transcription factors, bHLH34 and bHLH104, regulate iron homeostasis in Arabidopsis thaliana[J]. Plant Physiol, 170(4): 2478-2493.

Li Y, Lu CK, Li CY, et al., 2021. IRON MAN interacts with BRUTUS to maintain iron homeostasis in Arabidopsis[J]. Proc Natl Acad Sci USA, 118(39): e2109063118.

Liang G, Zhang H, Lou D, et al., 2016. Selection of highly efficient sgRNAs for CRISPR/Cas9-based plant genome editing[J]. Sci Rep, 6(1): 21451.

Mao QQ, Guan MY, Lu KX, et al., 2014. Inhibition of nitrate transporter 1.1-controlled nitrate uptake reduces cadmium uptake in Arabidopsis[J]. Plant Physiol, 166(2): 934-U730.

Matsubayashi Y, 2014. Posttranslationally modified small-peptide signals in plants[J]. Annu Rev Plant Biol, 65(1): 385-413.

Meng X, Li W, Shen R, et al., 2022. Ectopic expression of IMA small peptide genes confers tolerance to cadmium stress in Arabidopsis through activating the iron deficiency response[J]. J Hazard Mat, 422(1): 126913.

Nightingale ER, 1959. Phenomenological theory of ion solvation-effective radii of hydrated ions[J]. J Phys Chem, 63(9): 1381-1387.

Schutzendubel A, Polle A, 2002. Plant responses to abiotic stresses: heavy metal induced oxidative stress and protection by mycorrhization[J]. J Exp Bot, 53(372): 1351-1365.

Sebastian A, Prasad MN, 2016. Iron plaque decreases cadmium accumulation in Oryza sativa L. and serves as a source of iron[J]. Plant Biol, 18(6): 1008-1015.

Shao G, Chen M, Wang W, et al., 2007. Iron nutrition affects cadmium accumulation and toxicity in rice plants[J]. Plant Growth Regul, 53(1): 33-42.

Takahashi F, Hanada K, Kondo T, et al., 2019. Hormone-like peptides and small coding genes in plant stress signaling and development[J]. Curr Opin Plant Biol, 51(1): 88-95.

Takahashi R, Ishimaru Y, Senoura T, et al., 2011. The OsNRAMP1 iron transporter is involved in Cd accumulation in rice[J]. J Exp Bot, 62(14): 4843-4850.

Tavormina P, De Coninck B, Nikonorova N, et al., 2015. The plant peptidome: an expanding repertoire of structural features and biological functions[J]. Plant Cell, 27(8): 2095-2118.

Vert G, Grotz N, Dedaldechamp F, et al., 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth[J]. Plant Cell, 14(6): 1223-1233.

Wei B, Yang L, 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China[J]. Microchem J, 94(2): 99-107.

Wu H, Chen C, Du J, et al., 2012. Co-overexpression FIT with AtbHLH38 or AtbHLH39 in Arabidopsis-enhanced cadmium tolerance via increased cadmium sequestration in roots and improved iron homeostasis of shoots[J]. Plant Physiol, 158(2): 790-800.

Xu SS, Lin SZ, Lai ZX, 2015. Cadmium impairs iron homeostasis in Arabidopsis thaliana by increasing the polysaccharide contents and the iron-binding capacity of root cell walls[J]. Plant Soil, 392(1): 71-85.

Yao X, Cai Y, Yu D, et al., 2018. bHLH104 confers tolerance to cadmium stress in Arabidopsis thaliana[J]. J Integr Plant Biol, 60(8): 691-702.

Yoshihara T, Hodoshima H, Miyano Y, et al., 2006. Cadmium inducible Fe deficiency responses observed from macro and molecular views in tobacco plants[J]. Plant Cell Rep, 25(4): 365-373.

Yuan Y, Wu H, Wang N, et al., 2008. FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in Arabidopsis[J]. Cell Res, 18(3): 385-397.

Zhang J, Liu B, Li M, et al., 2015. The bHLH transcription factor bHLH104 interacts with IAA-LEUCINE RESISTANT3 and modulates iron homeostasis in Arabidopsis[J]. Plant Cell, 27(3): 787-805.

Zhu YX, Du WX, Fang XZ, et al., 2020. Knockdown of BTS may provide a new strategy to improve cadmium-phytoremediation efficiency by improving iron status in plants[J]. J Hazard Mat, 384(1): 121473.