We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Improving Rice Grain Quality by Enhancing Accumulation of Iron and Zinc While Minimizing Cadmium and Lead

Lei Gao and Jie Xiong

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.72826>

Abstract

Iron (Fe) and zinc (Zn) are important trace elements for people's health around the globe. A lot of people, especially children and woman, are suffering from malnutrition caused by Fe and/or Zn deficiency. The deficiency is more pronounced in some parts of Africa and Asia due to low income, which makes it difficult to afford meat or sea foods that are rich in Fe and Zn. Biofortification of Fe and Zn in rice is the most economical and convenient way to supplement these important minerals in the diet of poor people. However, besides Fe and Zn, rice also can accumulate heavy metals, such as cadmium (Cd) and lead (Pb), which are harmful to people, especially for kids' health. Previous researches have shown that there are connections and discrepancies for metal absorption, translocation, and accumulation in rice. So it is imperative to review these issues. This chapter compares the physiological and molecular mechanisms of Fe, Zn, Cd, and Pb uptake, mobilization, and accumulation in rice and discusses the progress and strategies for not only increasing Fe/Zn but also decreasing Cd/Zn accumulation in rice.

Keywords: biofortification, heavy metal, iron, zinc, cadmium, lead, stress

1. Introduction

Metal elements, such as Fe, Zn, Mn and Cu, are essential for living organisms and humans' growth. Various metal nutrients supplied by food contributed to maintain metabolism normally. Unfortunately, iron (Fe) and zinc (Zn), as the most important metal elements, are present in low quantities of staple food, such as rice and wheat [1]. What is worse is that, in some parts of Africa and Asia, people even cannot afford enough food for their kids and families. Fe deficiency is one of the leading risk factors of disability and death worldwide. It is estimated to affect two billion

people in the world [2]. Also, it has been estimated that about 30% population of the world suffers from Zn deficiency [3]. Fe-deficiency anemia can impair cognitive and physical development in children and the reduction of daily productivity in adults. Adequate Zn nutrition is also important for the growth of children, immune function, and neurobehavioral development [4]. Thus, Fe and Zn deficiency has emerged as a major and common problem for the health of humans [5, 6].

Rice is the staple food and provides energy to almost half of the world's population, especially in Asia and Africa. Thus, increasing Fe/Zn content of rice has a great potential to mitigate widespread Fe/Zn deficiency problem in humans [7]. Therefore, it is essential to understand the mechanisms through which rice uptakes, mobilizes, and accumulates Fe/Zn. In response to Fe deficiency, higher plants have developed two strategies for acquiring Fe from the rhizosphere [8, 9]. Strategy I is employed in nongraminaceous plants, and Fe(III) is reduced to soluble Fe(II) through activating membrane-bound Fe(III)-chelate reductases, then the reduced Fe(II) was transported into cytoplasm via Fe(II) transporters [10]. In contrast, Strategy II is only applied by graminaceous plants, such as rice and wheat. The root of strategy II plants secretes phytosiderophores (PSs) to rhizosphere and chelates Fe(III) to form Fe(III)-PS complexes. Subsequently, the Fe(III)-PS complexes are transported via specific plasma membrane transporters [7]. Rice not only employs strategy II to acquire Fe from rhizosphere but also utilizes strategy I-like system to uptake Fe(II) directly [10]. Besides Fe uptake, mugineic acid (MA) family also plays crucial roles in chelating Zn from rhizosphere, followed by uptake of Zn-PS complexes via specific plasma membrane transporters [7]. Moreover, Zn can be ionized as Zn(II) and directly enters into root [11]. In spite of rice can apply specific strategies to acquire Fe and Zn, these mechanisms have limited accessibility to resource poor people faced with Fe and Zn deficiency from certain areas of the world. To deal with limited Fe/Zn and improve human Fe/Zn nutritional status, rice with enhanced Fe/Zn absorption will be an effective method for populations consuming rice as their staple foods.

Besides necessary metal elements, such as Fe and Zn, rice also absorbs and accumulates toxic metals such as cadmium (Cd) and lead (Pb), which are harmful for both rice and humans. Cd enters into environment, such as soil and river, mainly through industrial activities or fertilizers [12]. As a highly mobile and soluble metal [13, 14], Cd exposure causes crops yield reduction and does harm to humans' health even at low concentrations [15]. Due to daily consumption, Cd in rice grains poses a latent health problem to humans through food chains and leads to chronic toxicity. The outbreak of "Itai-Itai disease" in the mid-twentieth century in Japan is due to consumption of Cd-contaminated rice [16]. A person with "Itai-Itai" has symptoms of weakness and softening of the bones. Even in recent years, Cd exposure in general Japanese population can be as high as 3–4 mg kg⁻¹ body weight every week [17]. The directly observable toxic symptoms of Cd on plants are as follows: reduced rate of transpiration and photosynthesis, growth retardation and declining metabolic activities [15]. In response to Cd toxicity, plants also have evolved several protective mechanisms against Cd toxicity, including avoidance and tolerance strategies [18]. Plants can prevent Cd from entering into plant cells, which is referred to as avoidance strategy. Cell walls serve as the first barrier against Cd entrance [15, 19]. Root exudates, which are majorly consisted of sugars, proteins and organic acids are secreted from root to soil to combine with Cd, keeping Cd apart from root [20, 21]. After entered into the cells, the abilities of resistance to Cd stress are referred to as tolerance

strategy [15]. In rice, phytochelatins (PC) acting as Cd chelator plays a key role in Cd detoxification [22]. PC chelates Cd in the cytosol and forms complexes with Cd. Then the Cd-PCs complexes are sequestered in the vacuoles via specific transporters located at tonoplast [23, 24].

Pb after arsenic (As) is ranked as the second most harmful element due to its occurrence, toxicity, and exposure potential [25]. Although Pb occurs naturally only in small amounts within the Earth's crust [26], a large amount of industrial activities are the primary sources of Pb in soil [27]. Pb is released into soil in general forms of Pb(II), lead oxide and lead-metal oxyanion, among which Pb(II) is the most common form [27]. Pb stress can produce reactive oxygen species (ROS) and trigger some antioxidative enzymes, accompanied by the increased level of lipid peroxidation [25, 27]. In rice, Pb toxicity reduces leaf chlorophyll and nitrogen content and increases antioxidative enzymes. Its subsequent translocation to grain causes a great threat to humans' health [28, 29]. High levels of Pb can cause brain and kidney damage, accompanied with central nervous prostration [27]. Therefore, higher plants possess preventive mechanisms against Pb. Similar to Cd detoxification, cell walls, and compartmentalization in vacuoles have been suggested as an important detoxification mechanism [27].

Many researches show that the mechanisms of Fe/Zn/Cd/Pb uptake and accumulation in rice share commons in some aspects as a result of similar entry routes (transporters) within rice cells. However, an increasing number of studies discovered distinct pathways and mechanisms of Fe/Zn/Cd/Pb uptake and accumulation in rice recently. In this chapter, we mainly systematically elaborate and compare physiological and cellular mechanisms of Fe/Zn/Cd/Pb uptake and accumulation in rice. In addition, we also review the mechanisms of maintaining Fe/Zn homeostasis and Cd/Pb detoxification in rice. Effects of fertilizers on Fe/Zn/Cd/Pb accumulation in rice are also discussed. Finally, we enumerate various approaches for reducing grain Cd/Pb accumulation and enhancing Fe/Zn content in rice.

2. Uptake of Fe/Zn and Cd/Pb from rhizosphere to root in rice

Mobility and availability of metals from soil are controlled by three following factors: (1) soil conditions (upland or flooded soil, soil solution pH); (2) mineralization (ionization and complex formation); and (3) uptake transporters [11].

Although Fe in acidic soil is ionized as $\text{Fe}^{2+}/\text{Fe}^{3+}$ and easily utilized by plants, Fe in aerobic alkaline soil is immobilized as $\text{Fe}(\text{OH})_3$. Rice absorbs Fe^{3+} via strategy II. In strategy II, *S*-adenosyl-L-methionine (SAM) is catalyzed by nicotianamine synthase (NAS) and produces nicotianamine (NA), which is an intermediate for the biosynthesis of MA family and a vital substance of nicotianamine aminotransferase (NAAT) [6]. Three rice NAS genes, *OsNAS1*, *OsNAS2*, and *OsNAS3*, have been identified to play different roles in Fe uptake and translocation in rice several years ago [30]. NAAT is a critical enzyme converting NA to 2'-deoxymugineic acid (DMA). Six rice NAAT genes (*OsNAAT1*–6) have been identified, but *OsNAAT1* was the only one highly upregulated under Fe deficiency, suggesting that *OsNAAT1* rather than *OsNAAT2*–6 encodes the unique functional enzyme possessing NAAT activity [31]. Cheng et al. [10] demonstrated that *NAAT1* mutant was not able to produce DMA and take up Fe(III) efficiently.

In rice, gene that encodes DMA efflux transporters (OsTOM1) is highly expressed under Fe deficiency stress [32]. TOM1 transporter localizes at plasma membrane and mediates DMA secretion to rhizosphere, followed by Fe(III)-DMA complexes formation [32]. *Yellow stripe 1* (YS1) gene that encodes Fe(III)-MAs transporters was first acquired in maize [33], and YS1-like (*OsYSL*) genes in rice have been subsequently identified over decades. *OsYSL15* has been demonstrated to be upregulated in rice root and shoot under Fe deficiency to transport Fe(III)-DMA complexes [34]. In addition, *OsYSL* genes that encode transporters are also involved in Fe translocation within rice [35, 36]. Once transported into cytosol, Fe(III)-DMA is reduced by ascorbate to form Fe(II)-NA [37]. NA is not only an important intermediate for the biosynthesis of MAs but also a significant metal chelator that can take part in translocation of Fe within plants [38]. Fe may be excreted to the xylem in the form of Fe(II)-NA and shift to make complexes predominantly with citrate (Fe_2Cit_2 , Fe_3Cit_3) and some with DMA [39]. The excretion of citrate from the root cells to the xylem is partly operated by OsFRDL1 (rice ferric reductase defective1-like) to enhance Fe-transport in the xylem as Fe(III)-citrate complexes [40].

In addition to Fe(III)-DMA uptake, rice also absorbs Fe(II) via iron-regulated transporter 1 (OsIRT1) and natural resistance-associated macrophage protein 1 (OsNRAMP1) under flooded conditions [16]. Seven rice *NRAMP* genes have been identified so far [41]. Recent research indicated that plasma membrane-localized protocatechuic acid (PCA) transporter and phenolic efflux zero1/2 (PEZ1/2) also participated in Fe uptake [42]. Such transporters played a role in absorbing apoplasmic precipitated Fe by secreting phenolics like PCA or caffeic acid. Suppression of *PEZ1/2* expression resulted in reduced Fe concentrations [42, 43].

Zn in both drained and flooded soil is largely ionized as (Zn^{2+}) though some Zn may be bound to organic substances and immobilized as Zn-sulfide (ZnS) in the anaerobic layer [7]. Zn-regulated transporters and iron-regulated transporters like protein (ZIP) family participate in Zn uptake [44]. OsZIP1-8 transporters have been characterized for Zn uptake and translocation in rice [7]. OsZIP1, OsZIP3, and OsZIP4 are required for Zn acquisition from rhizosphere [45]. OsZIP1 and OsZIP3 are mainly located in vascular bundles and epidermal cells, while OsZIP4 is located in apical meristem and phloem cells [45]. Under Zn deficiency stress, expression of *OsZIP1*, *OsZIP3*, and *OsZIP4* are upregulated [44, 45]. In addition, iron-regulated transporters (OsIRT) are also involved in Zn uptake [11]. OsIRT1 is characterized for Zn uptake in rice besides Fe acquisition [46, 47]. After transported into cytosol, Zn can be sequestered into the vacuoles via transporter OsZIP1 [11]. OsZIP1 is located to tonoplast and mediates influx of Zn into vacuoles [11]. MAs are also characterized for their role in Zn uptake in plants [7].

In comparison with Fe and Zn, Cd in paddy alkaline soil is present in the immobilized forms of CdCO_3 and humic acid-bound Cd [48]. Cd is immobilized as Cd-sulfide (CdS) and colloids-bound Cd in flooded soil [49]. Cd in drained acidic soil is ionized as Cd^{2+} , and drainage converts CdS to Cd^{2+} dramatically, increasing its availability to plants [11]. Cd uptake from rhizosphere is a dose-dependent process and exhibits saturable kinetic characteristics in rice [16, 50]. After analyzing the kinetics of Cd uptake by root in rice, Fujimaki et al. [50] suggested that uptake rate of Cd was proportional to Cd concentration in the culture solution within range from 0.05 to 100 nM, demonstrating a linear relationship between uptake rate and Cd concentration in a certain range. Ishikawa et al. [51] suggested that this kinetic

characteristic of Cd uptake could be mediated by transporters. In fact, entrance of Cd into root cells via transporter OsNRAMP5 or OsIRT1 has been proved, and OsNRAMP5 is predominantly applied [52, 53]. *OsNRAMP5* expression is identified in root epidermis, exodermis, and outer layers of the cortex as well as in tissues around the xylem [54]. Knockout of *OsNRAMP5* reduces Cd accumulation both in straw and grains slightly [4]. Slamet-Loedin et al. [4] also proposed that downregulation of *OsNRAMP5* is a preferential strategy to decrease Cd uptake by root. OsNRAMP5 not only mediates Cd uptake but also regulates manganese (Mn) uptake and has relatively a minor effect on Fe uptake under Fe starvation [54]. In addition, higher expression of *OsNRAMP1* in root could enhance Cd accumulation in shoot of rice, indicating that OsNRAMP1 was also related with Cd uptake and transport [55].

When exposed to Cd contamination, rice supplied with Fe^{3+} generally represents weaker toxic symptoms than rice supplied with Fe^{2+} . The phenomenon is largely attributed to divalent metal transporters that are nonselective for Fe^{2+} uptake. Rice can uptake either Fe^{2+} or Cd^{2+} consequently (**Figure 1**). In contrast, Fe^{3+} transporters are selective for Fe^{3+} with no affinity for other divalent cations, which decreases Cd entrance into rice root to a great extent and reduces Cd toxicity accordingly (**Figure 1**).

Figure 1. Schematic diagram of Fe and Cd uptake mechanisms in rice root. OsNRAMP1 and OsIRT1 can uptake either divalent ion Fe^{2+} or Cd^{2+} . NA is catalyzed by NAAT1 and finally forms DMA, which is secreted into rhizosphere via transporter OsTOM1 and chelates with Fe^{3+} to form complex. The complex is transported into cell via transporter OsYSL15.

After influx into cytosol, Cd is sequestered into the vacuoles via transporter OsHMA3 [56] and transiently stored in the form of complexes [15]. This pathway decreases Cd mobility in the cytosol and translocation from root to shoot [15, 57]. Enhancement of OsHMA3 activity has been found to increase storage of Cd in root and decrease the transport of Cd to the shoot and the final accumulation of Cd in rice grains [23]. *OsHMA3* is mainly expressed in root [24], and OsHMA3 localized at tonoplast belongs to P_{1B} -ATPases [58]. A high rate of root-to-shoot transport and subsequent accumulation in the grains of ^{107}Cd , which was administered from a culture solution, was observed in OsHMA3-depleted rice lines [51].

Lead forms various complexes with soil components, and only small parts of the lead present as these complexes in the soil solution are phytoavailable [59]. In soil, Pb may occur as a free metal ion or make complexes with inorganic constituents, such as HCO_3^- , CO_3^{2-} , SO_4^{2-} , and Cl^- . Pb also may exist as organic ligands with amino acids, fulvic acids, and humic acids [59]. Lead behavior in soil is mainly controlled by factors, such as pH [60], redox conditions [61], cation-exchange capacity, and organic and inorganic ligand levels [62]. Once adsorbed onto the rhizoderm roots surface, Pb may enter into the root passively, followed by translocating water streams while the mechanism by which Pb enters into root at the molecular level is still unknown. It is suggested that Pb enters into the root through several pathways, and a particular pathway is through ionic channels [59]. Several authors have demonstrated that Ca^{2+} -permeable channels are the main pathway by which Pb enters into root [63, 64]. Ca^{2+} from rhizosphere will compete with Pb^{2+} for common uptake position [25].

3. Translocation of Fe/Zn and Cd/Pb in rice

Following uptake by root, Fe, Zn and Cd are transported to shoot via xylem and phloem, where a large amount of vascular bundles exist [11]. This radial transport system includes symplasmic and apoplasmic pathways, but the former pathway is predominantly utilized as a result of impediment by Casparian strips occurring in apoplasmic pathway [65]. After Fe(II)-NA formation in the cytosol, Fe(II)-NA is transported to xylem and exchanges NA with citrate, transforming into Fe(III)-citrate preferentially [39, 40]. Fe in the xylem is largely in the form of Fe-citrate and then allocated to all leaves, whereas Fe in the phloem is mainly bound to DMA, citrate, and proteins [11]. The translocation of citrate from root pericycle cells to xylem is mediated by ferric reductase defective 1-like transporter OsFRDL1 [40].

Transportation of metals from plant root to shoot requires movement through the xylem [66] and is probably driven by transpiration [67]. Fe, Zn, Pb, and their chemical forms are in rice xylem and phloem saps, and phloem loading is the first step. OsYSL2 plays a role in Fe distribution in the phloem, localizing at the plasma membrane and is responsible for Fe(II)-NA or Mn(II)-NA transport, but not for Fe(III)-DMA transport [68]. Nozoye et al. [32] proposed that the NA efflux transporters (ENA1/2) are responsible for the efflux of NA into xylem or intracellular compartments in order to redistribute Fe. Under Fe deficiency, both *OsYSL2* and *ENA1* are strongly induced [68, 69]. In addition to transporter OsYSL2, OsYSL15 is considered to transport Fe(III)-DMA for phloem trafficking and expressed in the phloem companion cells

[6, 35, 43]. Thereafter, Fe is delivered to grains via phloem in forms of Fe(III)-DMA or binds to some citrates and proteins [11].

The Zn chemical forms in xylem sap are free ions and Zn partially bound to unidentified chelators [40], while the Zn in phloem sap was dominantly bound to NA [70]. Obata and Kitagishi [71] indicated that some Zn in the xylem (transpiration stream) is transferred to the phloem at the vegetative nodes in addition to the mobilization of Zn from mature leaves in rice. Xylem transfer cells have been found in rice vegetative nodes, and localized metal transporters may support the active xylem-to-phloem transfer of xylem sap Zn [72] and Cd [73].

After entered into root cells, part of Cd present as Cd-phytochelatin (Cd-PC) complexes are sequestered in the vacuoles, and the others are transported to xylem mediated by OsHMA2 transporter in root pericycle cells [11, 74, 75]. In the phloem, Cd primarily bounds to specific proteins and slightly to thiol-compounds [76]. In contrast to Fe translocation that is mainly derived from leaves by remobilization, xylem-to-phloem transfer system of Cd mainly occurs at the nodes [50]. In rice nodes, the diffuse vascular bundles (DVBs) that encircle the enlarged elliptical vascular bundles (EVBs) are connected to the panicle [77]. A study demonstrated that Cd was predominantly transported toward the panicle instead of other tissues at the panicle-initiation stage through the nodes and ultimately reached grains by positron-emitting ^{107}Cd tracer imaging system (PETIS) [50]. Node I, the uppermost node, is connected to both flag leaf and panicles. Yamaguchi et al. [77] found that Cd concentration was higher in node I than in blade, culm and panicle due to the accumulation of Cd. In addition, a low-affinity cation transporter (OsLCT1), which is highly expressed in the node I, also takes part in Cd transport to grains [16].

4. Effects of culture managements on Fe/Zn and Cd/Pb accumulation in rice grains

In rice seeds, Fe localizes to dorsal vascular bundle, aleurone layer and endosperm, and it localizes to the scutellum and vascular bundle of the scutellum of embryo [78]. Zn is distributed to all parts of the seed with a significantly high value for the aleurone layer and embryo [79]. Low Fe and Zn contents in rice are often restricted due to low available pools of Fe or Zn in soil. Enriching Fe or Zn concentration in grains through either fertilization or water management is referred to as agronomic biofortification, which is a short-term strategy for complementing the breeding programs.

Fe is abundant in mineral soil, but Fe deficiency still can occur in aerobic condition [80]. The major problem with Fe uptake is solubility. Fe in the soil (usually in the form of Fe^{2+} , either chelated or as a sulfate salt) is easily converted to unavailable Fe^{3+} under aerobic condition. Thus, application of Fe as fertilizer is not an effective strategy for increasing rice seed Fe [81]. Otherwise, foliar application is a better option to overcome Fe deficiency, increasing grains Fe and its bioavailability in rice [82]. In contrast, as soil changes from aerobic to anaerobic conditions after flooding, Fe-oxides are dissolved when the Fe^{3+} is reduced to Fe^{2+} , which weakens the oxide stability and increases its water solubility [83]. In fact, irrigation management in rice strongly influences soil redox potential, which affects the availability of Fe, so flooded soil nearly always has sufficient Fe for rice uptake [4].

Zn status and content in soil are the dominant factors restricting Zn content of rice seeds, followed by rice genotypes and fertilizers [84]. In aerobic condition, Zn mainly presents in soil in the form of ion Zn^{2+} . The application of Zn as fertilizer is effective in promoting rice growth and also in the fortification of rice with Zn [85, 86]. However, the availability of Zn decreases with flooding due to precipitation as insoluble zinc sulfide [87] or as insoluble carbonates mixtures [83]. Positive effects of soil Zn fertilization on grains Zn have been noticed primarily with aerobic water management [84]. In addition, foliar Zn application compared to soil Zn fertilization has been more effective in improving grains Zn concentration in flooded condition [88].

Although foliar application of Fe or Zn is more effective than soil application for increasing Fe or Zn content in rice grains, the efficiency of foliar applied Fe or Zn also depends on the application stages [89]. Late season foliar application of Zn or Fe at flowering or at early grain filling stage is more effective in improving grain Zn or Fe, respectively, than early season application [90, 91]. Although the level of Zn in grains is positively related with Fe, research showed that foliar fertilization of combined Fe and Zn fertilizers enhanced both grain Fe and Zn content without any antagonistic effects [82], indicating that fertilization of one element does not affect the grains concentration of the others [82, 92]. Totally, in order to increase both Fe and Zn content in rice grains under anaerobic or flooded conditions, the most effective fertilization strategy is a combination of foliar Zn and Fe spray soon after flowering or at early grain filling stage [4]. HarvestZinc Fertilizer Project started in 2008 and aimed at assessing the potential of Zn fertilizer in order to increase Zn content in cereal grains, especially in wheat and rice.

N fertilizer application has been reported to be related with Fe and Zn content in rice grains. Optimized N fertilizer application could increase grains Fe and Zn content in several crop species, including rice under sufficient Zn supply [92–94]. The reason is suggested as follows: (1) N nutrition promotes protein synthesis, which is a major sink for Fe and Zn [92]; (2) N nutrition enhances the expression of Zn and Fe transporter proteins, such as ZIP family transporters [92]; (3) N nutrition enhances the production of N compounds, such as NA and DMA [95]; (4) N nutrition increases Fe and Zn accumulation time by increasing vegetative growth and grain filling periods [4]. In contrast, N fertilizer can decrease rice grains Zn content under low Zn condition by increased biomass production and enhanced biological dilution [94]. In summary, optimized N fertilization application in rice production is very important to regulate Fe and Zn accumulation.

As a result of similar physical and chemical characteristics of Zn and Cd [96], Cd is mainly present as free Cd^{2+} in soil under aerobic condition regardless of soil redox potential [97], and the effect of flooding on Zn mobilization is indirect rather than direct compared with Fe. Cadmium in acidic soil is ionized as Cd^+ [48] and moves toward root system and translocates within plants, resulting in Cd accumulation eventually [98]. In previous reports, phosphate (P) fertilizer was thought to increase rice Cd accumulation [99, 100]. Because Cd emerges in the rock phosphate used for P fertilizer production, P fertilizers generally contain significant amounts of Cd [98]. Nowadays, these relatively high-Cd phosphate rock sources have been avoided in the fertilizer. Sarwar et al. [98] reported that mono-ammonium-phosphate (MAP) could enhance Cd solubility and uptake by lowering soil pH. However, Bolan et al. [12] reported that P fertilizer can reduce Cd solubility by insoluble Cd formation such as $\text{Cd}(\text{OH})_2$ or $\text{Cd}_3(\text{PO}_4)_2$. Yang et al. [101] proposed that P deprivation decreased rice Cd uptake by competitively increasing

Fe uptake and accumulation. P deprivation also enhances the sensitivity to Cd in rice plants by inhibiting biomass accumulation and reducing PCs synthesis deprivation.

An increasing number of evidences show that different N fertilizer forms and content affect Cd accumulation in rice. Sarwar et al. [98] reported that enhanced N application increased biomass production and reduced Cd toxicity to some extent due to dilution effect. N application increased soluble protein that could bind mobile Cd to immobile form. Different N fertilizer forms also have relationships with Cd uptake and accumulation in rice [102]. NH_4^+ -containing fertilizer is considered to contribute to enhance Cd uptake [98]. NH_4^+ -containing fertilizers acidify rhizosphere by proton excretion from root cells, exchanging with NH_4^+ and leading to low pH in soil [103]. In low pH soil, Cd moves toward root system and translocates within plants, resulting in Cd accumulation. In addition, NH_4^+ can trigger cell membrane depolarization and lead to influx of NH_4^+ into root cells, which accelerates translocation of Cd from root to shoot though this mechanism reduces Cd uptake in a certain way [98, 103]. In contrast, NO_3^- -containing fertilizer causes simultaneous NO_3^- and proton absorption by root cells, leading to high pH [99], and cell membrane polarization caused by nitrate can produce Cd detoxification mechanism [98]. Nevertheless, Xie et al. [104] found that plants supplied with NO_3^- accumulated more Cd than NH_4^+ treatment by *Thlaspi caerulescens* in hydroponic experiment, suggesting that effects of NH_4^+ and NO_3^- on Cd uptake are not simply attributed to rhizosphere pH transformation or charge distribution of cell membrane. Yang et al. [105] found that rice fed with excess NO_3^- not only enhanced Fe uptake but also increased Cd uptake by upregulating the expression of *OsIRT1*. In addition, Wangstrand et al. [106] once proposed that application of N fertilizer was dependent on different growth stages and recommended that more N fertilizer should be applied at the vegetative stage while less N doses should be applied during the grain filling stage.

Besides P and N, other fertilizers are also related with Cd accumulation in rice. Fe is reported to remarkably increase Cd concentration in root and shoot of rice [107]. In contrast, a peculiar mechanism against Cd stress by application of Fe fertilizer is iron plague (IP) formation [96]. IP can serve as a barrier and prevent Cd from entering into root cells, resulting in reduced Cd accumulation while enhanced Fe concentration in rice [108]. Si application can reduce mobilization of Cd due to increased pH in soil [98], and complexes formation of Si with Cd is another mechanism for alleviating Cd toxicity in rice [96]. Application of S fertilizer may decrease Cd toxicity in the form of insoluble CdS, by which reduces mobility of Cd in soil [109]. S also participates in GSH and PCs biosynthesis. S increases Cd tolerance by forming Cd-PCs complexes and being transported into vacuoles in rice cells [96, 98, 110].

Pb accumulation in rice was wildly reported in Southeast Asian countries, such as China. Many researchers reported the toxic effects of Pb on rice growth and mineral absorption [111], but researches on reducing Pb accumulation in rice grains by water and fertilizer managements are still limited. Hu et al. [112] reported that Selenium (Se) application reduced Pb concentration in rice tissues but had no significant effect on Pb accumulation in brown rice grains. Soil remediation methods are applied to reduce Cd/Pb toxicity to some extent, including soil removal, replacement, inversion and flooded condition before and after heading [41, 113], but it is not easy to apply. In summary, more work is still needed to explore the effect of water and fertilizer management on Pb accumulation in rice grains.

5. Breeding and transgenic approaches to increase Fe/Zn and reduce Cd/Pb accumulation in rice grains

Since 1992, researchers at International Rice Research Institute (IRRI) have evaluated the genetic variability of Fe [114] and Zn [115] concentration in rice grains. The range in Fe and Zn contents of 939 varieties tested in one study were 7.5–24.4 $\mu\text{g g}^{-1}$ for Fe, and 13.5–58.4 $\mu\text{g g}^{-1}$ for Zn [116]. Among these 939 varieties, high grains Fe and Zn concentrations were identified, including Jalmagna, Zuchem, and Xua Bue Nuo [116]. HarvestPlus Challenge Program launched in 2004 supports biofortification of staple food crops, including rice for increased Fe, Zn, and vitamin A. However, iron biofortification of rice based on conventional breeding has met with only marginal success. The iron level achieved to date is still too low to address the required target level set by HarvestPlus (around 14 $\mu\text{g g}^{-1}$), indicating that iron biofortification in rice remains a challenge [117]. In contrast, a number of varieties of biofortified zinc in rice and wheat are now available or being tested in countries all over the world, including India, Bangladesh, and Pakistan. Complementing the traditional breeding efforts, modern transgenic technology provides perspectives for efficiently improving Fe and Zn content of rice grains to dietary significant level for humans' nutrition [118].

Recent attempts on the biofortification of Fe and Zn in rice grains using transgenic techniques have shown some positive results. Overexpression of the barley NA synthase gene *HvNAS1* in rice plants caused an increase in DMA and NA concentrations in root, shoot, and seed, accompanied with enhanced Fe, Zn, and Cu concentrations in grains [119]. Zheng et al. [120] indicated that biofortified rice with NA could efficiently enhance bioavailability by overexpression *OsNAS1* in rice endosperm. Alexander et al. [121] constructed three rice populations overexpressing *OsNAS1*, *OsNAS2* and *OsNAS3*. These constitutive overexpression of the *OsNAS* genes led to increased NA level, positively correlated with enhanced Zn concentration both in unpolished and polished grains, which reduces Zn nutrient loss to some extent due to polishing process. Goto et al. [122] demonstrated that high level of Fe in rice endosperm could be acquired by overexpression of ferritin. Swamy et al. [123] suggested that overexpression of the ferritin gene *OsFer2* in basmati rice (Pusa Sugandh II) was observed to accumulate higher levels of Fe and Zn. Combination of upregulated expression of ferritin with overproduction of NA can significantly enhance Fe and Zn content [88]. In addition, manipulation of specific transporters involved in Fe/Zn uptake and translocation is also considered to be promising approach for enhancing Fe/Zn content. Ishimaru et al. [68] introduced *OsYSL2* mediated by sucrose transporter (*OsSUT1*) promoter into rice plants due to location of *OsSUT1* around endosperm, resulting in high concentration of Fe in polished rice. Overexpression of the Fe transporter gene *OsIRT1* or *OsYSL15*, the Fe deficiency-inducible bHLH transcription factor *OsIRO2*, and knockdown of the vacuolar Fe transporter gene *OsVIT1* or *OsVIT2*, were regarded as an effective approaches to increase the Fe concentration of seeds [124]. Overexpression of *OsHMA3* enhanced the uptake of Zn by upregulating the ZIP family genes in the root [125]. *OsHMA2* was involved in loading of Zn to the developing tissues in rice [75]. Quantitative trait locus (QTL) analysis is a useful approach to identify responsible genes for the respective transport processes [126]. Anuradna et al. [127] identified QTLs and candidate genes for Fe/Zn transport in rice seeds. *OsYSL1* and *OsMTP1* are responsible for Fe transport, while *OsARD2*, *OsIRT1*, *OsNAS1*, *OsNAS2* are responsible for

Zn transport. Ishikawa et al. [128] detected four QTLs (*qGZn9*, *qGZn10*, *qGZn2-1*, *qGZn2-2*) responsible for high Zn accumulation, and *qGZn9* showed the best effect, which provides valuable allele for breeding rice with high Zn level in grains.

Genotype dependence has been well observed for the accumulation of Cd in rice. More Cd accumulated in shoots and grains of *indica* cultivars than *japonica* cultivars [55, 129, 130]. Recently, Cd and Pb contents of 100 top Chinese rice cultivars were determined. The results also showed that *indica* accumulated more Cd than *japonica* [131]. Studies on rice screened for Cd-free, but Fe/Zn-rich cultivars have been an important issue in agricultural field. Significant efforts have been made on breeding of low-Cd accumulating rice cultivars in Japan, where Cd accumulation in rice grains has long been recognized as serious agricultural issues [41, 132]. Ishikawa et al. [132] identified and screened three low-Cd mutants (*lcd-kmt1*, *lcd-kmt2*, and *lcd-kmt3*) with *japonica* rice cultivar, Koshihikari, which acted as parent by the way of carbon ion-beam irradiation, showing that there were lower Cd concentration in grains of the three mutants than Koshihikari wide type (WT). Such three low-Cd mutants were attributed to mutations of *OsNRAMP5* responsible for Cd transport in rice by sequence analysis [132]. The three low-Cd mutants have different mutation sites in *OsNRAMP5*. An insertion of transposon *mPingA1*, which was activated by ion beam and preferred to insert into exon of *OsNRAMP5*, was identified in *lcd-kmt1*, resulting in nonfunction of *OsNRAMP5* and decreased Cd accumulation in grains [132]. Similar results were observed in *lcd-kmt2* and *lcd-kmt3* due to a single-base pair deletion and a large deletion in *OsNRAMP5*, respectively [132]. Meanwhile, Ishikawa et al. [132] proposed that *lcd-kmt1* and *lcd-kmt2* were more promising for breeding program according to agronomic traits as a consequence of earlier heading and smaller plant size than Koshihikari WT in *lcd-kmt3* [132]. In addition, Abe et al. [133] developed a novel population composed of 46 chromosome segment substitution lines (CSSLs), in which LAC23 served as donor segments and were substituted into Koshihikari. LAC23 could result in lower grain-to-straw ratio than Koshihikari [133]. Therefore, cultivars containing LAC23 performed low Cd content in grains [133]. As for breeding Fe/Zn-rich cultivars, Olive et al. [134] bred high level of ferritin cultivars with rice mega variety IR64 as background and introducing ferritin into endosperm increased Fe content in grains [132, 133]. IR64 mutants obtained from sodium azide treatment were reported to have high Zn level [123]. Booyaves et al. [135] expressed Arabidopsis IRT1 (*AtIRT1*) in high-iron NFP rice lines, which expressed *NICOTIANAMINE SYNTHASE* (*AtNAS1*) and *FERRITIN*, enhancing Fe contents in both unpolished and polished grains.

In addition, QTL analysis was also applied to identify responsible genes for Cd transport. QTL for Cd concentration in Anjana Dhan (*indica* rice cultivar) is identified on chromosome 7, responsive gene for which is *OsHMA3* [23, 55, 126]. Abe et al. [136] introduced a non-functional allele of *OsHMA3* from Jarian (*indica* rice cultivar) into Koshihikari (*japonica* rice cultivar) by marker-assisted selection, and these plants showed reduced Cd uptake from soil. Suppression of *OsLCT1* expression can decrease grains Cd accumulation by RNAi without influencing nutrient accumulation. On the contrary, Fe content in the brown rice is remarkably higher [16], suggesting that RNAi-mediated *OsLCT1* suppression in rice is a promising approach to establish “high Fe but low-Cd-rice.” Furthermore, T-DNA-mediated *OsLCD* knockout mutant showed reduced grain Cd accumulation while having no negative effect on grain yield. Thus, the *lcd* mutant might be a probable mutant line for further research [137].

Breeding low Pb cultivars is also considered to reduce Pb contamination. Developing rice cultivars with low Pb mobilization within root and translocation toward aerial parts to the minimum extent may be a better option to cultivate rice in Pb tainted soils. Li et al. [138] screened three cultivars (Tianyou196, Wufengyou2168, and Guinongzhan) with low Pb level in brown rice. Furthermore, Ashraf et al. [139] compared Meixiangzhan (MXZ-2), Xiangyaxiangzhan(XYXZ), Guixiangzhan (GXZ), Basmati-385 (B-385), and Nongxiang-18 (NX-18) to four different Pb concentrations, indicating that GXZ proved better able to tolerate Pb stress than all other rice cultivars, which are therefore suggested for use in future breeding programs for paddy fields contaminated by Pb.

6. Conclusions and perspectives

Fe and Zn are essential nutrients for humans, but Cd and Pb are toxic at high levels for humans. All these metals accumulated in the grains of rice, a staple cereal worldwide. Compared with Pb, significant progress has been made in investigating the mechanisms for Fe, Zn, and Cd uptake and accumulation in rice grains. These basic discoveries provide us with the increasing possibility to establish high Fe/Zn and low Cd/Pb rice. Here, we summarized a strategy scheme for producing biofortified Fe/Zn but low Cd/Pb rice cultivars as follows (Figure 2). On the one hand, scientists have screened or bred nontransgenic rice cultivars with high Fe/Zn or/and low

Figure 2. Schematic diagram of strategies for enhancing accumulation of iron and zinc while minimizing cadmium and lead in rice. Based on functional QTLs or genes, scientists screen or breed nontransgenic rice cultivars with high Fe/Zn or/and low Cd/Pb in grains. Modern transgenic technology provides perspectives for efficiently improving Fe/Zn content and decreasing Cd/Pb content in rice grains. Water and fertilizers management are also significantly related with increased Fe/Zn and decreased Cd/Pb in rice grains.

Cd/Pb in grains based on functional QTLs or genes. These cultivars show no agriculturally or economically adverse traits and can be applied sooner. On the other hand, modern transgenic technology provides perspectives for efficiently improving Fe/Zn content and decreasing Cd/Pb content in rice grains to dietary significant levels for humans' nutrition (As to Pb, more researches on QTL and genes still needed). Besides improving rice seeds, water and fertilizer management is also significantly related with increased Fe/Zn and decreased Cd/Pb in rice grains. More studies are still needed to optimize irrigation time, fertilizer categories, dosage, and application stages. In addition, although it is available to establish rice cultivars with high Fe or Zn content, or establish rice cultivars with low Cd or Pb separately, interactions among these metals need to be better understood, and more steps are still needed to cultivate rice with all these merits and without decreasing rice production.

Acknowledgements

This research was supported by Zhejiang Provincial Natural Science Foundation of China under Grant No. LY18C130011 and LY15C130007, Science Foundation of Zhejiang Sci-Tech University, Foundation of Zhejiang Provincial Top Key Discipline of Biology, and Foundation of Zhejiang Provincial Key Discipline of Botany.

Author details

Lei Gao and Jie Xiong*

*Address all correspondence to: jiexiong@zju.edu.cn

College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou, People's Republic of China

References

- [1] Gómez-Galera S, Rojas E, Sudhakar D, Zhu C, Pelacho AM, Capell T, Christou P. Critical evaluation of strategies for mineral fortification of staple food crops. *Transgenic Research*. 2010;**19**:165-180. DOI: 10.1007/s11248-009-9311-y
- [2] Sperotto RA, Ricachenevsky FK, Waldow Vde A, Fett JP. Iron biofortification in rice: It's a long way to the top. *Plant Science*. 2012;**190**:24-39. DOI: 10.1016/j.plantsci.2012.03.004
- [3] Welch RM. The impact of mineral nutrients in food crops on global human health. In: Horst WJ et al., editors. *Progress in Plant Nutrition: Plenary Lectures of the XIV International Plant Nutrition Colloquium*. Developments in Plant and Soil Sciences. Dordrecht: Springer; 2002
- [4] Slamet-Loedin IH, Johnson-Beebout SE, Impa S, Tsakirpaloglou N. Enriching rice with Zn and Fe while minimizing cd risk. *Frontiers in Plant Science*. 2015;**6**:121. DOI: 10.3389/fpls.2015.00121

- [5] Alloway B. Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health*. 2009;**31**:537-548. DOI: 10.1007/s10653-009-9255-4
- [6] Kobayashi T, Nakanishi H, Nishizawa NK. Recent insights into iron homeostasis and their application in graminaceous crops. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*. 2010;**86**:900-913. DOI: 10.2183/pjab.86.900
- [7] Bashir K, Ishimaru Y, Nishizawa NK. Molecular mechanisms of zinc uptake and translocation in rice. *Plant and Soil*. 2012;**361**:189-201. DOI: 10.1007/s11104-012-1240-5
- [8] Conte SS, Walker EL. Transporters contributing to iron trafficking in plants. *Molecular Plant*. 2011;**4**:464-476. DOI: 10.1093/mp/ssr015
- [9] Kobayashi T, Nishizawa NK. Iron uptake, translocation, and regulation in higher plants. *Annual Review of Plant Biology*. 2012;**63**:131-152. DOI: 10.1146/annurev-arplant-042811-105522
- [10] Cheng L, Wang F, Shou H, Huang F, Zheng L, He F, Li J, Zhao FJ, Ueno D, Ma JF, Wu P. Mutation in nicotianamine aminotransferase stimulated the Fe(II) acquisition system and led to iron accumulation in rice. *Plant Physiology*. 2007;**145**:1647-1657. DOI: 10.1104/pp.107.107912
- [11] Yoneyama T, Ishikawa S, Fujimaki S. Route and regulation of zinc, cadmium, and iron transport in rice plants (*Oryza sativa* L.) during vegetative growth and grain filling: Metal transporters, metal speciation, grain cd reduction and Zn and Fe biofortification. *International Journal of Molecular Sciences*. 2015;**16**:19111-19129. DOI: 10.3390/ijms160819111
- [12] Bolan N, Mahimairaja S, Kunhikrishnan A, Naidu R. Sorption-bioavailability nexus of arsenic and cadmium in variable-charge soils. *Journal of Hazardous Materials*. 2013;**261**:725-732. DOI: 10.1016/j.jhazmat.2012.09.074
- [13] He S, He Z, Yang X, Stoffella PJ, Baligar VC. Chapter four – Soil biogeochemistry, plant physiology, and phytoremediation of cadmium-contaminated soils. *Advances in Agronomy*. 2015;**134**:135-225. DOI: 10.1016/bs.agron.2015.06.005
- [14] Song W, Chen S, Chen J, Song N, Li N, Liu B. Variation of cd concentration in various rice cultivars and derivation of cadmium toxicity thresholds for paddy soil by species-sensitivity distribution. *Journal of Integrative Agriculture*. 2015;**14**:1845-1854. DOI: 10.1016/S2095-3119(14)60926-6
- [15] Choppala G, Saifullah Bolan N, Bibi S, Iqbal M, Rengel Z, Kunhikrishnan A, Ashwath N, Yong SO. Cellular mechanisms in higher plants governing tolerance to cadmium toxicity. *Critical Reviews in Plant Sciences*. 2014;**33**:374-391. DOI: 10.1080/07352689.2014.903747
- [16] Uraguchi S, Kamiya T, Sakamoto T, Kasai K, Sato Y, Nagamura Y, Yoshida A, Kyoizuka J, Ishikawa S, Fujiwara T. Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**:20959-22064. DOI: 10.1073/pnas.1116531109
- [17] Tsukahara T, Ezaki T, Moriguchi J, Furuki K, Shimbo S, Matsuda-Inoguchi N, Ikeda M. Rice as the most influential source of cadmium intake among general Japanese population. *Science of the Total Environment*. 2003;**305**:41-51. DOI: 10.1016/S0048-9697(02)00475-8

- [18] DalCorso G, Farinati S, Furini A. Regulatory networks of cadmium stress in plants. *Plant Signaling & Behavior*. 2010;**5**:663-667. DOI: 10.4161/psb.5.6.11425
- [19] Lang I, Wernitznig S. Sequestration at the cell wall and plasma membrane facilitates zinc tolerance in the moss *Pohlia drummondii*. *Environmental and Experimental Botany*. 2011;**74**:186-193. DOI: 10.1016/j.envexpbot.2011.05.018
- [20] Schwab AP, He Y, Banks MK. The influence of organic ligands on the retention of lead in soil. *Chemosphere*. 2005;**61**:856-866. DOI: 10.1016/j.chemosphere.2005.04.098
- [21] Dong J, Mao WH, Zhang GP, FB W, Cai Y. Root excretion and plant tolerance to cadmium toxicity – A review. *Plant, Soil and Environment*. 2007;**53**:193-200
- [22] Yadav R, Arora P, Kumar S, Chaudhury A. Perspectives for genetic engineering of poplars for enhanced phytoremediation abilities. *Ecotoxicology*. 2010;**19**:1574-1588. DOI: 10.1007/s10646-010-0543-7
- [23] Ueno D, Yamaji N, Kono I, Huang CF, Ando T, Yano M, Ma JF. Gene limiting cadmium accumulation in rice. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:16500-16505. DOI: 10.1073/pnas.1005396107
- [24] Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, Kawamoto T, Katou K, Kodama I, Sakurai K, Takahashi H, Satoh-Nagasawa N, Watanabe A, Fujimura T, Akagi H. OsHMA3, a P1B-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *The New Phytologist*. 2011;**189**:190-199. DOI: 10.1111/j.1469-8137.2010.03459.x
- [25] Ashraf U, Kanu AS, Mo Z, Hussain S, Anjum SA, Khan I, Abbas RN, Tang X. Lead toxicity in rice: Effects, mechanisms, and mitigation strategies – A mini review. *Environmental Science and Pollution Research International*. 2015;**22**:18318-11832. DOI: 10.1007/s11356-015-5463-x
- [26] Ashraf MA, Wajid A, Mahmood K, Maah MJ, Yusoff I. Removal of heavy metals from aqueous solution by using mango biomass. *African Journal of Biotechnology*. 2011;**10**: 2163-2177
- [27] Gupta DK, Huang HG, Corpas FJ. Lead tolerance in plants: Strategies for phytoremediation. *Environmental Science and Pollution Research International*. 2013;**20**:2150-2161. DOI: 10.1007/s11356-013-1485-4
- [28] Davis JM, Elias RW, Grant LD. Current issues in human lead exposure and regulation of lead. *Neurotoxicology*. 1993;**14**:15-27
- [29] Liu J, Li K, Xu J, Zhang Z, Ma T, Lu X, Yang J, Zhu Q. Lead toxicity, uptake, and translocation in different rice cultivars. *Plant Science*. 2003;**165**:793-802. DOI: 10.1016/S0168-9452(03)00273-5
- [30] Inoue H, Higuchi K, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. Three rice nicotianamine synthase genes, *OsNAS1*, *OsNAS2*, and *OsNAS3* are expressed in cells involved in long-distance transport of iron and differentially regulated by iron. *The Plant Journal*. 2003;**36**:366-381. DOI: 10.1046/j.1365-313X.2003.01878.x

- [31] Inoue H, Takahashi M, Kobayashi T, Suzuki M, Nakanishi H, Mori S, Nishizawa NK. Identification and localisation of the rice nicotianamine aminotransferase gene *OsNAAT1* expression suggests the site of phytosiderophore synthesis in rice. *Plant Molecular Biology*. 2008;**66**:193-203. DOI: 10.1007/s11103-007-9262-8
- [32] Nozoye T, Nagasaka S, Kobayashi T, Takahashi M, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK. Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *The Journal of Biological Chemistry*. 2011;**286**:5446-5454. DOI: 10.1074/jbc.M110.180026
- [33] Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL. Maize *yellow stripe1* encodes a membrane protein directly involved in Fe(III) uptake. *Nature*. 2001;**409**:346-349. DOI: 10.1038/35053080
- [34] Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK. 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. *The Journal of Biological Chemistry*. 2009;**284**:3470-3479. DOI: 10.1074/jbc.M806042200
- [35] Kakei Y, Ishimaru Y, Kobayashi T, Yamakawa T, Nakanishi H, Nishizawa NK. OsYSL16 plays a role in the allocation of iron. *Plant Molecular Biology*. 2012;**79**:583-594. DOI: 10.1007/s11103-012-9930-1
- [36] Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *The Plant Journal*. 2004;**39**:415-424. DOI: 10.1111/j.1365-313X.2004.02146.x
- [37] Weber G, von Wirén N, Hayen H. Investigation of ascorbate-mediated iron release from ferric phytosiderophores in the presence of nicotianamine. *Biometals*. 2008;**21**:503-513. DOI: 10.1007/s10534-008-9137-8
- [38] Takahashi H, Kosaka M, Watanabe Y, Nakade K, Fukuyama Y. Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. *Bioorganic & Medicinal Chemistry*. 2003;**11**:1781-1788. DOI: 10.1016/S0968-0896(02)00666-1
- [39] Ariga T, Hazama K, Yanagisawa S, Yoneyama T. Chemical forms of iron in xylem sap from graminaceous and non-graminaceous plants. *Soil Science and Plant Nutrition*. 2014;**60**:460-469. DOI: 10.1080/00380768.2014.922406
- [40] Yokosho K, Yamaji N, Ueno D, Mitani N, Ma JF. OsFRDL1 is a citrate transporter required for efficient translocation of iron in rice. *Plant Physiology*. 2009;**149**:297-305. DOI: 10.1104/pp.108.128132
- [41] Uraguchi S, Fujiwara T. Cadmium transport and tolerance in rice: Perspectives for reducing grain cadmium accumulation. *Rice*. 2012;**5**:5. DOI: 10.1186/1939-8433-5-5
- [42] Ishimaru Y, Kakei Y, Shimo H, Bashir K, Sato Y, Sato Y, Uozumi N, Nakanishi H, Nishizawa NK. A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. *The Journal of Biological Chemistry*. 2011;**286**:24649-24655. DOI: 10.1074/jbc.M111.221168

- [43] Kobayashi T, Nakanishi Itai R, Nishizawa NK. Iron deficiency responses in rice roots. *Rice*. 2014;**7**:27. DOI: 10.1186/s12284-014-0027-0
- [44] Ishimaru Y, Suzuki M, Kobayashi T, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. OsZIP4, a novel zinc-regulated zinc transporter in rice. *Journal of Experimental Botany*. 2005;**56**:3207-3214. DOI: 10.1093/jxb/eri317
- [45] Ramesh SA, Shin R, Eide DJ, Schachtman DP. Differential metal selectivity and gene expression of two zinc transporters from rice. *Plant Physiology*. 2003;**133**:126-134. DOI: 10.1104/pp.103.026815
- [46] Lee S, An G. Over-expression of OsIRT1 leads to increased iron and zinc accumulations in rice. *Plant, Cell & Environment*. 2009;**32**:408-416. DOI: 10.1111/j.1365-3040.2009.01935.x
- [47] Nouet C, Motte P, Hanikenne M. Chloroplastic and mitochondrial metal homeostasis. *Trends in Plant Science*. 2011;**16**:395-404. DOI: 10.1016/j.tplants.2011.03.005
- [48] Khaokaew S, Chaney RL, Landrot G, Gindervogel M, Sparks DL. Speciation and release kinetics of cadmium in an alkaline paddy soil under various flooding periods and draining conditions. *Environmental Science & Technology*. 2011;**45**:4249-4255. DOI: 10.1021/es103971y
- [49] Hashimoto Y, Yamaguchi N. Chemical speciation of cadmium and sulfur K-edge XANES spectroscopy in flooded paddy soils amended with zerovalent iron. *Soil Science Society of America Journal*. 2013;**77**:1189. DOI: 10.2136/sssaj2013.01.0038
- [50] Fujimaki S, Suzui N, Ishioka NS, Kawachi N, Ito S, Chino M, Nakamura S. Tracing cadmium from culture to spikelet: Noninvasive imaging and quantitative characterization of absorption, transport, and accumulation of cadmium in an intact rice plant. *Plant Physiology*. 2010;**152**:1796-1806. DOI: 10.1104/pp.109.151035
- [51] Ishikawa S, Suzui N, Ito-Tanabata S, Ishii S, Igura M, Abe T, Kuramata M, Kawachi N, Fujimaki S. Real-time imaging and analysis of differences in cadmium dynamics in rice cultivars (*Oryza sativa*) using positron-emitting ¹⁰⁷Cd tracer. *BMC Plant Biology*. 2011;**11**:172. DOI: 10.1186/1471-2229-11-172
- [52] Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa NK. Iron deficiency enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1 and OsIRT2 in rice. *Soil Science and Plant Nutrition*. 2006;**52**:464-469. DOI: 10.1111/j.1747-0765.2006.00055.x
- [53] Sasaki A, Yamaji N, Yokosho K, Ma JF. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *The Plant Cell*. 2012;**24**:2155-2167. DOI: 10.1105/tpc
- [54] Ishimaru Y, Takahashi R, Bashir K, Shimo H, Senoura T, Sugimoto K, Ono K, Yano M, Ishikawa S, Arao T, Nakanishi H, Nishizawa NK. Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Scientific Reports*. 2012;**2**:286. DOI: 10.1038/srep00286
- [55] Takahashi R, Ishimaru Y, Senoura T, Shimo H, Ishikawa S, Arao T, Nakanishi H, Nishizawa NK. The OsNRAMP1 iron transporter is involved in cd accumulation in rice. *Journal of Experimental Botany*. 2011;**62**:4843-4850. DOI: 10.1093/jxb/err136

- [56] Takahashi R, Bashir K, Ishimaru Y, Nishizawa NK, Nakanishi H. The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. *Plant Signaling & Behavior*. 2012;**7**:1605-1607. DOI: 10.4161/psb.22454
- [57] Shahid M, Dumat C, Khalid S, Niazi NK. Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system. *Reviews of Environmental Contamination and Toxicology*. 2017;**241**:73-137. DOI: 10.1007/398_2016_8
- [58] Williams LE, Mills RF. P₁B-ATPases – An ancient family of transition metal pumps with diverse functions in plants. *Trends in Plant Science*. 2005;**10**:491-502. DOI: 10.1016/j.tplants.2005.08.008
- [59] Pourrut B, Shahid M, Dumat C, Winterton P, Pinelli E. Lead uptake, toxicity, and detoxification in plants. Vol. 213. In: Whitacre D, editor. *Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews)*. New York: Springer; 2011. pp. 113-136
- [60] Lawal OS, Sanni AR, Ajayi IA, Rabiou OO. Equilibrium, thermodynamic and kinetic studies for the biosorption of aqueous lead(II) ions onto the seed husk of *Calophyllum inophyllum*. *Journal of Hazardous Materials*. 2010;**177**:829-835. DOI: 10.1016/j.jhazmat.2009.12.108
- [61] Tabelin CB, Igarashi T. Mechanisms of arsenic and lead release from hydrothermally altered rock. *Journal of Hazardous Materials*. 2009;**169**:980-990. DOI: 10.1016/j.jhazmat.2009.04.049
- [62] Shahid M, Pinelli E, Pourrut B, Silvestre J, Dumat C. Lead-induced genotoxicity to *Vicia faba* L. roots in relation with metal cell uptake and initial speciation. *Ecotoxicology and Environmental Safety*. 2011;**74**:78-84. DOI: 10.1016/j.ecoenv.2010.08.037
- [63] Wang HH, Shan XQ, Wen B, Owens G, Fang J, Zhang SZ. Effect of indole-3-acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and the relevant antioxidant response. *Environmental and Experimental Botany*. 2007;**61**:246-253. DOI: 10.1016/j.envexpbot.2007.06.004
- [64] Pourrut B, Perchet G, Silvestre J, Cecchi M, Guiesse M, Pinelli E. Potential role of NADPH-oxidase in early steps of lead-induced oxidative burst in *Vicia faba* roots. *Journal of Plant Physiology*. 2008;**165**:571-579. DOI: 10.1016/j.jplph.2007.07.016
- [65] Enstone DE, Peterson CA, Ma F. Root endodermis and exodermis: Structure, function, and responses to the environment. *Journal of Plant Growth Regulation*. 2002;**21**:335-351. DOI: 10.1007/s00344-003-0002-2
- [66] Verbruggen N, Hermans C, Schat H. Mechanisms to cope with arsenic or cadmium excess in plants. *Current Opinion in Plant Biology*. 2009;**12**:364-372. DOI: 10.1016/j.pbi.2009.05.001
- [67] Liao YC, Chien SW, Wang MC, Shen Y, Hung PL, Das B. Effect of transpiration on Pb uptake by lettuce and on water soluble low molecular weight organic acids in rhizosphere. *Chemosphere*. 2006;**65**:343-351. DOI: 10.1016/j.chemosphere.2006.02.010
- [68] Ishimaru Y, Masuda H, Bashir K, Inoue H, Tsukamoto T, Takahashi M, Nakanishi H, Aoki N, Hirose T, Ohsugi R, Nishizawa NK. Rice metal-nicotianamine transporter,

- OsYSL2, is required for the long-distance transport of iron and manganese. *The Plant Journal*. 2010;**62**:379-390. DOI: 10.1111/j.1365-313X.2010.04158.x
- [69] Ogo Y, Kakei Y, Itai RN, Kobayashi T, Nakanishi H, Takahashi H, Nakazono M, Nishizawa NK. Spatial transcriptomes of iron-deficient and cadmium-stressed rice. *The New Phytologist*. 2014;**201**:781-794. DOI: 10.1111/nph.12577
- [70] Nishiyama R, Kato M, Nagata S, Yanagisawa S, Yoneyama T. Identification of Zn-nicotianamine and Fe-2'-Deoxymugineic acid in the phloem sap from rice plants (*Oryza sativa* L.). *Plant & Cell Physiology*. 2012;**53**:381-390. DOI: 10.1093/pcp/pcr188
- [71] Obata H, Kitagishi K. Investigation on pathway of Zn in vegetative node of rice plants by autoradiography: Behavior of zinc in rice plants (III). *Japanese Journal of Soil Science and Plant Nutrition*. 1980;**51**:297-301
- [72] Nishiyama R, Tanoi K, Yanagisawa S, Yoneyama T. Quantification of zinc transport via the phloem to the grain in rice plants (*Oryza sativa* L.) at early grain-filling by a combination of mathematical modeling and ⁶⁵Zn tracing. *Soil Science & Plant Nutrition*. 2013;**59**:750-755. DOI: 10.1080/00380768.2013.819774
- [73] Rodda MS, Li G, Reid RJ. The timing of grain Cd accumulation in rice plants: The relative importance of remobilisation within the plant and root Cd uptake post-flowering. *Plant and Soil*. 2011;**347**:105-114. DOI: 10.1007/s11104-011-0829-4
- [74] Takahashi R, Ishimaru Y, Shimo H, Ogo Y, Senoura T, Nishizawa NK, Nakanishi H. The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant, Cell & Environment*. 2012;**35**:1948-1957. DOI: 10.1111/j.1365-3040.2012.02527.x
- [75] Yamaji N, Xia J, Mitaniueno N, Yokosho K, Ma JF. Preferential delivery of zinc to developing tissues in rice is mediated by P-type heavy metal ATPase OsHMA2. *Plant Physiology*. 2013;**162**:927-939. DOI: 10.1104/pp.113.216564
- [76] White PJ, Broadley MR. Physiological limits to zinc biofortification of edible crops. *Frontiers in Plant Science*. 2011;**2**:80. DOI: 10.3389/fpls.2011.00080
- [77] Yamaguchi N, Ishikawa S, Abe T, Baba K, Arao T, Terada Y. Role of the node in controlling traffic of cadmium, zinc, and manganese in rice. *Journal of Experimental Botany*. 2012;**63**:2729-2737. DOI: 10.1093/jxb/err455
- [78] Bashir K, Takahashi R, Nakanishi H, Nishizawa NK. The road to micronutrient biofortification of rice: Progress and prospects. *Frontiers in Plant Science*. 2013;**4**:15. DOI: 10.3389/fpls.2013.00015
- [79] Takahashi M, Nozoye T, Kitajima N, Fukuda N, Hokura A, Terada Y, Nakai I, Ishimaru Y, Kobayashi T, Nakanishi H. In vivo analysis of metal distribution and expression of metal transporters in rice seed during germination process by microarray and X-ray fluorescence imaging of Fe, Zn, Mn, and Cu. *Plant and Soil*. 2009;**325**:39-51. DOI: 10.1007/s11104-009-0045-7
- [80] Zuo Y, Zhang F. Soil and crop management strategies to prevent iron deficiency in crops. *Plant and Soil*. 2010;**339**:83-95. DOI: 10.1007/s11104-010-0566-0

- [81] Briat JF, Ravet K, Arnaud N, Duc C, Boucherez J, Touraine B, Cellier F, Gaymard F. New insights into ferritin synthesis and function highlight a link between iron homeostasis and oxidative stress in plants. *Annals of Botany*. 2010;**105**:811-822. DOI: 10.1093/aob/mcp128
- [82] Wei Y, Shohag MJ, Yang X, Yibin Z. Effects of foliar iron application on iron concentration in polished rice grain and its bioavailability. *Journal of Agricultural and Food Chemistry*. 2012;**60**:11433-11439. DOI: 10.1021/jf3036462
- [83] Kirk G. *The Biogeochemistry of Submerged Soils*. West Sussex, England: Wiley; 2004
- [84] Wang YY, Wei YY, Dong LX, Lu LL, Feng Y, Zhang J, Pan FS, Yang XE. Improved yield and Zn accumulation for rice grain by Zn fertilization and optimized water management. *Journal of Zhejiang University. Science B*. 2014;**15**:365-374. DOI: 10.1631/jzus.B1300263
- [85] Neue HU, Quijano C, Senadhira D, Setter T. Strategies for dealing with micronutrient disorders and salinity in lowland rice systems. *Field Crops Research*. 1998;**56**:139-155. DOI: 10.1016/S0378-4290(97)00125-1
- [86] Yang X, Ye ZQ, Shi CH, Zhu ML, Graham RD. Genotypic differences in concentrations of iron, manganese, copper, and zinc in polished rice grains. *Journal of Plant Nutrition*. 1998;**21**:1453-1462. DOI: 10.1080/01904169809365495
- [87] Bostick BC, Hansel CM, Fendorf S. Seasonal fluctuations in zinc speciation within a contaminated wetland. *Environmental Science & Technology*. 2001;**35**:3823-3829. DOI: 10.1021/es010549d
- [88] Wirth J, Poletti S, Aeschlimann B, Yakandawala N, Drosse B, Osorio S, Tohge T, Fernie AR, Günther D, Gruissem W, Sautter C. Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. *Plant Biotechnology Journal*. 2009;**7**:631-644. DOI: 10.1111/j.1467-7652.2009.00430.x
- [89] Cakmak I. Enrichment of fertilizers with zinc: An excellent investment for human-ity and crop production in India. *Journal of Trace Elements in Medicine and Biology*. 2009;**23**:281-289. DOI: 10.1016/j.jtemb.2009.05.002
- [90] Phattarakul N, Rerkasem B, Li LJ, Wu LH, Zou CQ, Ram H, Sohu VS, Kang BS, Surek H, Kalayci M. Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant and Soil*. 2012;**361**:131-141. DOI: 10.1007/s11104-012-1211-x
- [91] Mabesa RL, Impa SM, Grewal D, Johnson-Beebout SE. Contrasting grain-Zn response of biofortification rice (*Oryza sativa* L.) breeding lines to foliar Zn application. *Field Crops Research*. 2013;**149**:223-233. DOI: 10.1016/j.fcr.2013.05.012
- [92] Cakmak I, Kalayci M, Kaya Y, Torun AA, Aydin N, Wang Y, Arisoy Z, Erdem H, Yazici A, Gokmen O, Ozturk L, Horst WJ. Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry*. 2010;**58**:9092-9102. DOI: 10.1021/jf101197h
- [93] Kutman UB, Yildiz B, Ozturk L, Cakmak I. Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. *Cereal Chemistry*. 2010; **87**:1-9. DOI: 10.1094/CCHEM-87-1-0001

- [94] Jin Z, Wu L, Wang M. Can iron and zinc in rice grains (*Oryza sativa* L.) be biofortified with nitrogen fertilisation under pot conditions? *Journal of Science and Food Agriculture*. 2008;**88**:1172-1177. DOI: 10.1002/jsfa.3194
- [95] Haydon MJ, Cobbett CS. Transporters of ligands for essential metal ions in plants. *The New Phytologist*. 2007;**174**:499-506. DOI: 10.1111/j.1469-8137.2007.02051.x
- [96] Imtiaz M, Rizwan MS, Mushtaq MA, Ashraf M, Shahzad SM, Yousaf B, Saeed DA, Rizwan M, Nawaz MA, Mehmood S, Silicon TS. Occurrence, uptake, transport and mechanisms of heavy metals, minerals and salinity enhanced tolerance in plants with future prospects: A review. *Journal of Environmental Management*. 2016;**183**:521-529. DOI: 10.1016/j.jenvman.2016.09.009
- [97] Du Laing G, Rinklebe J, Vandecasteele B, Meers E, Tack FM. Trace metal behaviour in estuarine and riverine floodplain soils and sediments: A review. *Science of the Total Environment*. 2009;**407**:3972-3985. DOI: 10.1016/j.scitotenv.2008.07.025
- [98] Sarwar N, Saifullah MSS, Zia MH, Naeem A, Bibi S, Farid G. Role of mineral nutrition in minimizing cadmium accumulation by plants. *Journal of the Science of Food and Agriculture*. 2010;**90**:925-937. DOI: 10.1002/jsfa.3916
- [99] Eriksson JE. Effects of nitrogen-containing fertilizers on solubility and plant uptake of cadmium. *Water, Air, & Soil Pollution*. 1990;**49**:355-368. DOI: 10.1007/BF00507075
- [100] Williams CH, David DJ. The effect of superphosphate on the cadmium content of soils and plants. *Soil Research*. 1973;**11**:43-56. DOI: 10.1071/sr9730043
- [101] Yang Y, Chen R, Fu G, Xiong J, Tao L. Phosphate deprivation decreases cadmium (cd) uptake but enhances sensitivity to cd by increasing iron (Fe) uptake and inhibiting phytochelatin synthesis in rice (*Oryza sativa*). *Acta Physiologiae Plantarum*. 2016;**38**:28. DOI: 10.1007/s11738-015-2055-9
- [102] Mitchell LG, Grant CA, Racz GJ. Effect of nitrogen application on concentration of cadmium and nutrient ions in soil solution and in durum wheat. *Canadian Journal of Soil Science*. 2000;**80**:107-115. DOI: 10.4141/S98-085
- [103] Zaccheo P, Crippa L, Pasta VDM. Ammonium nutrition as a strategy for cadmium mobilisation in the rhizosphere of sunflower. *Plant and Soil*. 2006;**283**:43-56. DOI: 10.1007/s11104-005-4791-x
- [104] Xie HL, Jiang RF, Zhang FS, Mcgrath SP, Zhao FJ. Effect of nitrogen form on the rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator *Thlaspi caerulescens*. *Plant and Soil*. 2009;**318**:205-215. DOI: 10.1007/s11104-008-9830-y
- [105] Yang Y, Xiong J, Chen R, Fu G, Chen T, Tao L. Excessive nitrate enhances cadmium (cd) uptake by up-regulating the expression of OsIRT1 in rice (*Oryza sativa*). *Environmental and Experimental Botany*. 2016;**122**:141-149. DOI: 10.1016/j.envexpbot.2015.10.001
- [106] Wangstrand H, Eriksson J, Oborn I. Cadmium concentration in winter wheat as affected by nitrogen fertilization. *European Journal of Agronomy*. 2007;**26**:209-214. DOI: 10.1016/j.eja.2006.09.010

- [107] Shao G, Chen M, Wang D, Xu C, Mou R, Cao Z, Zhang X. Using iron fertilizer to control cd accumulation in rice plants: A new promising technology. *Science in China. Series C, Life Sciences*. 2008;**51**:245-253. DOI: 10.1007/s11427-008-0031-y
- [108] Liu H, Zhang J, Christie P, Zhang F. Influence of iron plaque on uptake and accumulation of cd by rice (*Oryza sativa* L.) seedlings grown in soil. *Science of the Total Environment*. 2008;**394**:361-368. DOI: 10.1016/j.scitotenv.2008.02.004
- [109] Hassan MJ, Feng W, Ali S, Zhang G. Toxic effect of cadmium on rice as affected by nitrogen fertilizer form. *Plant and Soil*. 2005;**277**:359-365. DOI: 10.1007/s11104-005-8160-6
- [110] Cobbett CS. Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*. 2000;**123**:825-832. DOI: 10.1104/pp.123.3.825
- [111] Gopal R, Rizvi AH. Excess lead alters growth, metabolism and translocation of certain nutrients in radish. *Chemosphere*. 2008;**70**:1539-1544. DOI: 10.1016/j.chemosphere.2007.08.043
- [112] Hu Y, Norton GJ, Duan G, Huang Y, Liu Y. Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. *Plant and Soil*. 2014;**384**:131-140. DOI: 10.1007/s11104-014-2189-3
- [113] Arao T, Kawasaki A, Baba K, Mori S, Matsumoto S. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. *Environmental Science & Technology*. 2009;**43**:9361-9367. DOI: 10.1021/es9022738
- [114] Gregorio GB, Senadhira D, Htut H, Graham RD. Breeding for trace mineral density in rice. *Food and Nutrition Bulletin*. 2000;**21**:382-386. DOI: 10.1177/156482650002100407
- [115] Graham JF, Mccague C, Norton PR. Topography and nanomechanical properties of tribochemical films derived from zinc dialkyl and diaryl dithiophosphates. *Tribology Letters*. 1999;**6**:149-157. DOI: 10.1023/A:1019124026402
- [116] Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*. 2004;**55**:353-364. DOI: 10.1093/jxb/erh064
- [117] Clemens S. Zn and Fe biofortification: The right chemical environment for human bioavailability. *Plant Science*. 2014;**225**:52-57. DOI: 10.1016/j.plantsci.2014.05.014
- [118] Bhullar NK, Boonyaves K, Wang M, Sautter C. Iron and zinc enhancement in rice endosperm by targeted and synergistic action of genes. In: Preedy V, Srirajaskanthan R, Patel V, editors. *Handbook of Food Fortification and Health. Nutrition and Health*. New York, NY: Humana Press; 2013. pp. 293-230. DOI: 10.1007/978-1-4614-7076-2_23
- [119] Masuda H, Usuda K, Kobayashi T, Ishimaru Y, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Mori S, Nishizawa NK. Overexpression of the barley nicotianamine synthase gene *HvNAS1* increases iron and zinc concentrations in rice grains. *Rice*. 2009;**2**:155-166. DOI: 10.1007/s12284-009-9031-1

- [120] Zheng L, Cheng Z, Ai C, Jiang X, Bei X, Zheng Y, Glahn RP, Welch RM, Miller DD, Lei XG, Shou H. Nicotianamine, a novel enhancer of rice iron bioavailability to humans. *PLoS One*. 2010;**5**:e10190. DOI: 10.1371/journal.pone.0010190
- [121] Alexander ATJ, Bianca Kyriacou, Damien LC, Lorrnine C, James S, Enzo L, Mark T. Constitutive overexpression of the *OsNAS* gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. 2011;**6**:e24476. DOI: 10.1371/journal.pone.0024476
- [122] Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F. Iron fortification of rice seed by the soybean ferritin gene. *Nature Biotechnology*. 1999;**17**:282-286. DOI: 10.1038/7029
- [123] Swamy BPM, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P, Reinke R, Slamet-Loedin IH. Advances in breeding for high grain zinc in rice. *Rice*. 2016;**9**:49. DOI: 10.1186/s12284-016-0122-5
- [124] Masuda H, Aung MS, Nishizawa NK. Iron biofortification of rice using different transgenic approaches. *Rice*. 2013;**6**:40. DOI: 10.1186/1939-8433-6-40
- [125] Sasaki A, Yamaji N, Ma JF. Overexpression of *OsHMA3* enhances Cd tolerance and expression of Zn transporter genes in rice. *Journal of Experimental Botany*. 2014;**65**:6013-6021. DOI: 10.1093/jxb/eru340
- [126] Ishikawa S, Abe T, Kuramata M, Yamaguchi M, Ando T, Yamamoto T, Yano M. A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. *Journal of Experimental Botany*. 2010;**61**:923-934. DOI: 10.1093/jxb/erp360
- [127] Anuradha K, Agarwal S, Rao YV, Rao KV, Viraktamath BC, Sarla N. Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar×Swarna RILs. *Gene*. 2012;**508**:233-240. DOI: org/10.1016/j.gene.2012.07.054
- [128] Ishikawa R, Iwata M, Taniko K, Monden G, Miyazaki N, Om C, Tsujimura Y, Yoshida S, Ma JF, Ishii T. Detection of quantitative trait loci controlling grain zinc concentration using Australian wild rice, *Oryza meridionalis*, a potential genetic resource for biofortification of rice. *Plos One*. 2017;**12**:e0187224. DOI: org/10.1371/journal.pone.0187224
- [129] Arao T, Ae N. Genotypic variations in cadmium levels of rice grain. *Soil Science & Plant Nutrition*. 2003;**49**:473-479. DOI: 10.1080/00380768.2003.10410035
- [130] Ishikawa S, Ae N, Sugiyama M, Murakami M, Arao T. Genotypic variation in shoot cadmium concentration in rice and soybean in soils with different levels of cadmium contamination. *Soil Science & Plant Nutrition*. 2010;**51**:101-108. DOI: 10.1111/j.1747-0765.2005.tb00012.x
- [131] Xie LH, Tang SQ, Wei XJ, Shao GN, Jiao GA, Sheng ZH, Luo J, PS H. The cadmium and lead content of the grain produced by leading Chinese rice cultivars. *Food Chemistry*. 2017;**217**:217-224. DOI: 10.1016/j.foodchem.2016.08.099

- [132] Ishikawa S, Ishimaru Y, Igura M, Kuramata M, Abe T, Senoura T, Hase Y, Arao T, Nishizawa NK, Nakanishi H. Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**:19166-19171. DOI: 10.1073/pnas.1211132109
- [133] Abe T, Nonoue Y, Ono N, Omoteno M, Kuramata M, Fukuoka S, Yamamoto T, Yano M, Ishikawa S. Detection of QTLs to reduce cadmium content in rice grains using LAC23/Koshihikari chromosome segment substitution lines. *Breeding Science*. 2013;**63**:284-291. DOI: 10.1270/jsbbs.63.284
- [134] Oliva N, Chadha-Mohanty P, Poletti S, Abrigo E, Atienza G, Torrizo L, Garcia R, Dueñas CJ, Poncio MA, Balindong J, Manzanilla M, Montecillo F, Zaidem M, Barry G, Hervé P, Shou H, Slamet-Loedin IH. Large-scale production and evaluation of marker-free indica rice IR64 expressing phytoferritin genes. *Molecular Breeding*. 2014;**33**:23-37. DOI: 10.1007/s11032-013-9931-z
- [135] Boonyaves K, Gruissem W, Bhullar NK. NOD promoter-controlled *AtIRT1* expression functions synergistically with NAS and FERRITIN genes to increase iron in rice grains. *Plant Molecular Biology*. 2016;**90**:207-215. DOI: 10.1007/s11103-015-0404-0
- [136] Abe T, Fumio TS, Kojima Y, Ebitani T, Kuramata M, Yamamoto T, Yano M, Ishikawa S. Detection of a QTL for accumulating cd in rice that enables efficient cd phytoextraction from soil. *Breeding Science*. 2011;**61**:43-51. DOI: 10.1270/jsbbs.61.221
- [137] Shimo H, Ishimaru Y, An G, Yamakawa T, Nakanishi H, Nishizawa NK. Low cadmium (LCD), a novel gene related to cadmium tolerance and accumulation in rice. *Journal of Experimental Botany*. 2011;**62**:5727-5734. DOI: 10.1093/jxb/err300
- [138] Li B, Wang X, Qi XL, Huang L, Ye ZH. Identification of rice cultivars with low brown rice mixed cadmium and lead contents and their interactions with the micronutrients iron, zinc, nickel and manganese. *Journal of Environmental Sciences (China)*. 2012;**24**:1790-1798. DOI: 10.1016/S1001-0742(11)60972-8
- [139] Ashraf U, Hussain S, Anjum SA, Abbas F, Tanveer M, Noor MA, Tang XR. Alterations in growth, oxidative damage, and metal uptake of five aromatic rice cultivars under lead toxicity. *Plant Physiology and Biochemistry*. 2017;**115**:461-471. DOI: 10.1016/j.plaphy.2017.04.019