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

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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).

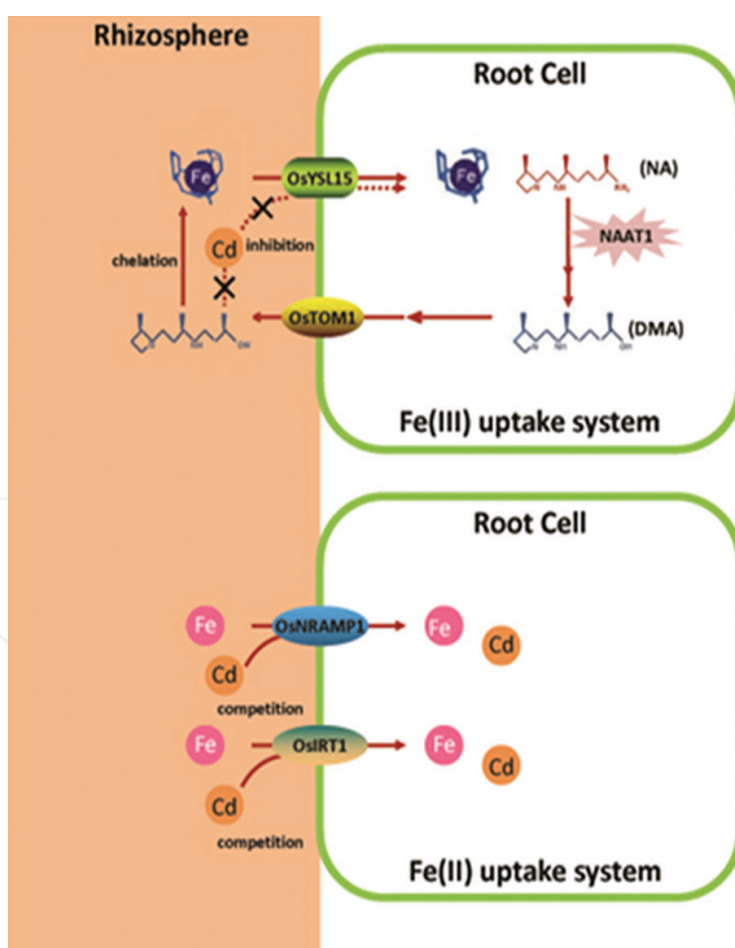


Figure 1. Schematic diagram of Fe and Cd uptake mechanisms in rice root. OsNRAMP1 and OsIRT1 can uptake either divalent ion Fe^{2+} or Cd^{2+} . NA is catalyzed by NAAT1 and finally forms DMA, which is secreted into rhizosphere via transporter OsTOM1 and chelates with Fe^{3+} to form complex. The complex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

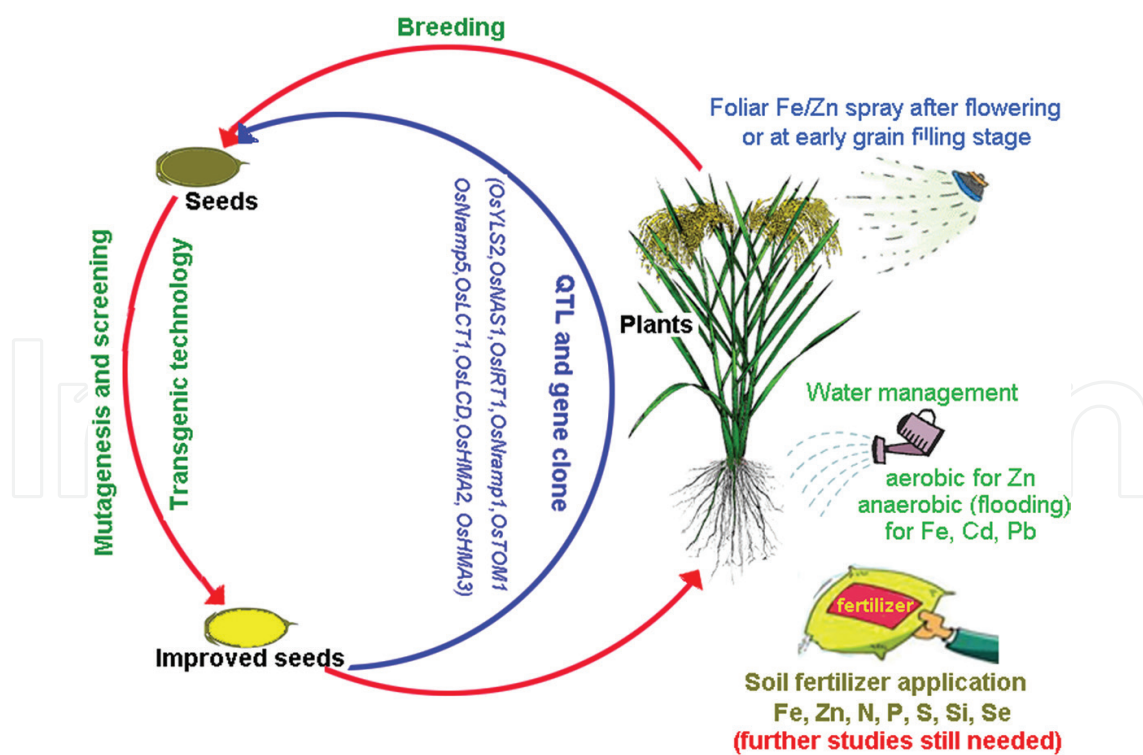


Figure 2. Schematic diagram of strategies for enhancing accumulation of iron and zinc while minimizing cadmium and lead in rice. Based on functional QTLs or genes, scientists screen or breed nontransgenic rice cultivars with high Fe/Zn or/and low Cd/Pb in grains. Modern transgenic technology provides 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.

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