Rice

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Comparison on cellular mechanisms of iron and cadmium accumulation in rice: prospects for cultivating Fe-rich but Cd-free rice

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Abstract

Iron (Fe) is essential for rice growth and humans consuming as their staple food but is often deficient because of insoluble Fe(III) in soil for rice growth and limited assimilation for human bodies, while cadmium (Cd) is non-essential and toxic for rice growth and humans if accumulating at high levels. Over-accumulated Cd can cause damage to human bodies. Selecting and breeding Fe-rich but Cd-free rice cultivars are ambitious, challenging and meaningful tasks for researchers. Although evidences show that the mechanisms of Fe/Cd uptake and accumulation in rice are common to some extent as a result of similar entry routes within rice, an increasing number of researchers have discovered distinct mechanisms between Fe/Cd uptake and accumulation in rice. This comprehensive review systematically elaborates and compares cellular mechanisms of Fe/Cd uptake and accumulation in rice, respectively. Mechanisms for maintaining Fe homeostasis and Cd detoxicification are also elucidated. Then, effects of different fertilizer management on Fe/Cd accumulation in rice are discussed. Finally, this review enumerates various approaches for reducing grain Cd accumulation and enhancing Fe content in rice. In summary, understanding of discrepant cellular mechanisms of Fe/Cd accumulation in rice provides guidance for cultivating Fe-fortified rice and has paved the way to develop rice that are tolerant to Cd stress, aiming at breeding Fe-rich but Cd-free rice.

Keywords: Iron, Cadmium, Biofortification, Mugineic acid, Phytosiderophores, Phytochelatins, Harvestplus, Fertilizer management

Review

Metal elements, such as Fe, Zn, Mn and Cu, are essential for living organisms and present as ions. Although there are abundant metal elements in the earth's crust, these ions, particularly Fe, are sparingly soluble under aerobic conditions in high pH or calcareous soils and are not bioavailable to plants (Takahashi et al. 2003). As a result, Fe deficiency is a widespread agricultural problem that causes plants growth retardation and restricts sources of nutrition from plants (e.g., rice, maize and barley) (Mori 1999; Kobayashi et al. 2010). In response to Fe deficiency, higher plants have developed two strategies for acquiring Fe from the rhizosphere (Conte and Walker, 2011; Kobayashi and Nishizawa, 2012). The application of strategy I is non-graminaceous plants, which includes the reduction of Fe(III) to soluble Fe(II) by activating membrane-bound Fe(III)-chelate reductases, followed by uptake of the reduced Fe(II) into cytoplasm via Fe(II) transporters (Cheng et al. 2007). Strategy II is employed only by graminaceous plants, such as rice. Roots can secrete phytosiderophores (PSs) that belongs to the muginneic acid (MA) family to rhizosphere and chelate Fe(III), followed by uptake of Fe(III)-PS complexes via specific plasma membrane transporters (Conte and Walker, 2011). Rice utilizes strategy II to acquire Fe from rhizosphere and also possesses strategy I-like

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system that can take in Fe(II) directly (Cheng et al. 2007). In spite of rice can apply specific strategies to acquire Fe, these mechanisms have limited accessibility to resource-poor people faced with Fe deficiency from certain areas of the world. To deal with limited Fe and improve human Fe nutritional status, biofortifying rice with enhanced Fe absorption will be an effective method for populations consuming rice as their staple food.

Cd is a toxic heavy metal and accumulation of Cd in rice grains poses a latent health problem to human. Cd in human body can lead to chronic toxicity. The outbreak of "Itai-Itai disease" in the mid-20th century in Japan is due to consumption of Cd-contaminated rice (Uraguchi et al. 2011). A person with "Itai-Itai" has symptoms of weakness and softening of the bones (Horiguchi et al. 2010). Cd enters into environment, such as soil and river mainly through industrial activities or fertilizers (Bolan et al. 2003). As a mobile and soluble metal, Cd causes crops yield reduction and does harm to human health even at low concentrations (Choppala et al. 2014). The primary effects on plants caused by Cd-induced toxic symptoms are as follows: reduced rate of transpiration and photosynthesis, growth retardation and declining metabolic activities (Choppala et al. 2014). In response to Cd toxicity, plants have evolved protective mechanisms against Cd toxicity, including "avoidance" and "tolerance" (DalCorso et al. 2010). "Avoidance", which means plants can prevent Cd from entering into cells and cell walls serve as the first parclose against Cd (Lang and Wernitznig, 2011; Choppala et al. 2014). Root exudates that majorly consist of sugars, proteins and organic acids are secreted from roots to soil, combining with Cd or keeping apart from roots (Schwab et al. 2005; Dong et al. 2007). After Cd inflows into cells, the abilities of resistance to Cd stress are referred to as "tolerance" (Choppala et al. 2014). A Cd chelator, phytochelatins (PC) plays a key role in Cd detoxification (Yadav et al. 2010). PC functions as chelating Cd in the cytosol and forming complexes with Cd. Complexes are sequestered in the vacuoles via specific transporters located at tonoplast (Ueno et al. 2010; Miyadate et al. 2011).

There is evidence that mechanisms of Fe/Cd uptake and accumulation in rice are common to some extent as a result of similar entry routes within rice. Nevertheless, an increasing number of researchers have discovered distinct mechanisms between Fe/Cd uptake and accumulation. This comprehensive review systematically elaborates and compares cellular mechanisms of Fe/Cd uptake and accumulation in rice at different stages, respectively. Mechanisms for maintaining Fe homeostasis and Cd detoxicification are elucidated. Effects of different fertilizer management on Fe/Cd accumulation in rice are discussed. Furthermore, this review enumerates various approaches for reducing grain Cd accumulation

and enhancing Fe content in rice. In summary, understanding of discrepant cellular mechanisms of Fe/Cd accumulation in rice provides guidance for cultivating Fe-fortified rice and has paved the way to develop rice that are tolerant to Cd stress, aiming at breeding Fe-rich but Cd-free cultivars.

Primary acquisition of Fe and Cd from rhizosphere to roots of rice

In strategy II of Fe acquisition, MAs are originated from S-adenosyl-l-L-methi-onine (SAM). SAM can be catalyzed by nicotianamine synthase (NAS) and produce nicotianamine (NA), which is an intermediate for the biosynthesis of MA family and a vital substance of nicotianamine aminotransferase (NAAT) (Kobayashi et al. 2010). Currently, three rice NAS genes, *OsNAS1*, *OsNAS2* and *OsNAS3* have been identified, playing different roles in Fe uptake and translocation (Inoue et al. 2003). NAAT is a critical enzyme in the biosynthesis of MAs that converts NA to 2'-deoxymugineic acid (DMA). Inoue et al. (2008) identified six rice *NAAT* genes (*OsNAAT1-6*), but only *OsNAAT1* was highly upregulated under Fe deficiency, suggesting that *OsNAAT1*, but not *OsNAAT2-6*, encodes the sole functional enzyme possessing NAAT activity. DMA chelates Fe(III) and then forms Fe(III)-DMA complexes, which are absorbed by root cells (Cheng et al. 2007). Cheng et al. (2007) demonstrated that *NAAT1* mutant was not able to produce DMA and take up Fe(III) efficiently.

Under Fe-deficiency stress, transporters related genes for Fe uptake and translocation are transcriptionally induced (Kobayashi et al. 2014). As for rice, gene encoding DMA efflux transporters (OsTOM1) is highly expressed in response to low Fe availability (Nozoye et al. 2011). *OsTOM1* encodes TOM1 transporter that localizes at plasma membrane and mediates DMA secretion to rhizosphere, followed by Fe(III)-DMA complexes formation (Nozoye et al. 2011) (Fig. 1a). *Yellow stripe 1 (YS1)* gene that encodes Fe(III)-MAs transporters was first acquired in maize. Maize *YS1* mutant presents interveinal chlorosis characteristic due to Fe deficiency (Curie et al. 2001). *YS1*-like (*OsYSL*) genes in rice have been subsequently identified over the decades, among which *OsYSL15* that transports Fe(III)-DMA is up-regulated in roots and shoots under Fe deficiency (Inoue et al. 2009). Fe(III)-DMA are absorbed via plasma membrane-bound OsYSL15 transporter (Inoue et al. 2009) (Fig. 1a). Furthermore, transporters that *OsYSL* genes encode are also involved in Fe translocation within rice (Koike et al. 2004; Kakei et al. 2012). Once inside the cytosol, Fe(III)-DMA can be reduced by ascorbate, forming Fe(II)-NA (Weber et al. 2008) (Fig. 1a). Hence, NA is not only an important intermediate for the biosynthesis of MAs, but

Fig. 1 Mechanisms of Fe/Cd uptake and translocation in rice. **a** Fe uptake from rhizosphere into root cells by specific root transporters. DMA is synthesized in cells and secreted into the rhizosphere by OsTOM1. DMA chelates rhizospheric Fe(III), forming Fe(III)-DMA complexes. Complexes are then taken up into root cells by OsYLS15. Roots also take up Fe(II) directly by metal transporters (OsIRT1/OsNRAMP1). **b** Cd is absorbed from rhizosphere into root cells mediated by OsIRT1 and OsNRAMP5. OsHMA3 plays a critical role in Cd compartmentalization into vacuoles in root cells. **c** Cd xylem loading in roots for translocation to shoots by OsHMA2, and Cd phloem loading for storage to grain sink. OsLCT1 and OsHMA2 mediate xylem-to-phloem transfer at nodes. **d** Fe xylem loading in roots for translocation to shoots and the remobilization of Fe through phloem from leaves for storage to grain sink. OsFRDL1, which is a citrate transporter localized at the root pericycle cells. OsFRDL1 loads citrate into the xylem and combines with Fe. ENA may be involved in efflux of NA into xylem. OsYSL2 then mediate Fe(II)-NA for phloem loading. OsTOM1 potentially participates in DMA transport, followed by mediating Fe(III)-DMA through OsYLS15. Furthermore, OsIRT1 directly transports Fe(II) in phloem companion cells of shoots. The encircled numerals represent the main localization of specific transporters. Right parts of the figure are adapted partially from Kobayashi et al. (2014) and Yoneyama et al. (2015)

also a significant metal chelator that can take part in translocation of Fe within plants (Takahashi et al. 2003).

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 (Takahashi et al. 2011) (Fig. 1a). Seven rice *NRAMP* genes have been identified so far (Uraguchi and Fujiwara, 2012).

The recent research indicated that plasma membrane-localized protocatechuic acid (PCA) transporter, phenolic efflux zero1/2 (PEZ1/2), also participated in Fe uptake (Ishimaru et al. 2011). Such transporter 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 (Ishimaru et al. 2011; Kobayashi et al. 2014).

In comparison, Cd uptake from rhizosphere is a dose-dependent process and exhibits saturable kinetic characteristics in rice (Fujimaki et al. 2010; Ishikawa et al. 2011). Fujimaki et al. (2010) analyzed the kinetics of Cd uptake by roots in rice and suggested uptake rate of Cd was proportional to Cd concentration in the culture solution within a range from 0.05 to 100 nM, demonstrating a linear relationship between uptake rate and Cd

concentration in a certain range. Ishikawa et al. (2011) suggested that this kinetic characteristic of Cd uptake could be mediated by transporters.

Cd enters into root cells via transporter OsNRAMP5 or OsIRT1 and OsNRAMP5 is predominantly applied (Nakanishi et al. 2006; Sasaki et al. 2012). *OsNRAMP5* expression is identified in roots epidermis, exodermis, and outer layers of the cortex as well as in tissues around the xylem (Ishimaru et al. 2012) (Fig. 1b). Knock-out of *OsNRAMP5* reduces Cd accumulation both in straw and grains slightly (Slamet-Loedin et al. 2015). Slamet-Loedin et al. (2015) also proposed that down-regulation of *OsN-RAMP5* is a preferential strategy to decrease Cd uptake by roots. OsNRAMP5 not only mediates Cd uptake, but also manganese (Mn) uptake and relatively minor effect on Fe uptake under Fe starvation (Ishimaru et al. 2012) (Table 1).

In addition, Takahashi et al. (2011) found that higher expression of *OsNRAMP1* in roots could enhance Cd accumulation in shoots of rice, indicating that OsNRAMP1 may take part in Cd uptake and transport besides Fe absorption (Takahashi et al. 2011). Consequently, such common characteristic of transporter-mediated acquisition mechanism paves the way for Cd accumulation in rice.

After influx of Cd into cytosol, one significant pathway of Cd is sequestered into the vacuole via transporter OsHMA3 (Takahashi et al. 2012a) and transiently stored in the form of complexes (Choppala et al. 2014) (Fig. 1b). This pathway decreases Cd mobility in the cytosol and translocation from roots to shoots (Choppala et al. 2014; Shahid et al. 2016). *OsHMA3* is mainly expressed in roots (Miyadate et al. 2011). OsHMA3 belongs to P_{1B}-ATPases and localizes at tonoplast (William and Mills, 2005). In

Table 1 Rice genes involved in Fe/Cd transport and induced status under Fe deficiency and Cd stress

Gene name	RAP ID	Function	Induced status under Fe deficiency and Cd stress[a]	References
NA/DMA biosynthesis for Fe(III)-DMA or Fe(II)-NA transport				
OsNAS1	Os03g0307300	Nicotianamine synthase	↑	Cheng et al. 2007
OsNAS2	Os03g0307200	Nicotianamine synthase	↑	Cheng et al. 2007
OsNAS3	Os07g0689600	Nicotianamine synthase	↑(root)↓(leaf)	Cheng et al. 2007
OsNAAT1	Os02g0306401	Nicotianamine aminotransferase	↑	Inoue et al. 2008
OsDMAS1	Os03g0237100	Deoxymugineic acid	↑	Kobayashi et al. 2014
Transporters for Fe/Cd uptake and translocation				
OsTOM1	Os11g0134900	DMA efflux transporter	↑	Nozoye et al. 2011
OsYSL15	Os02g0650300	Fe(III)-DMA transporter	↑	Inoue et al. 2009
OsYSL16	Os04g0542800	Fe(III)-DMA transporter	→	Kakei et al. 2012
PEZ1	Os03g0571900	Phenolics efflux transporter	—	Ishimaru et al. 2011
PEZ2	Os03g0572900	Phenolics efflux transporter	—	Ishimaru et al. 2012
OsIRT1	Os03g0667500	Ferrous Fe transporter	↑	Takahashi et al. 2011
OsIRT2	Os03g0667300	Ferrous Fe transporter	↑	Takahashi et al. 2011
OsNRAMP1	Os07g0258400	Ferrous Fe/Cd transporter	↑	Takahashi et al. 2011
OsNRAMP5	Os07g0257200	Ferrous Fe/Cd/Mn transporter	↑	Ishimaru et al. 2011
OsFRDL1	Os03g0216700	Citrate efflux transporter	→	Kobayashi et al. 2014
ENA1	Os11g0151500	NA efflux transporter	↑(root)↓(shoot)	Nozoye et al. 2011
ENA2	Os06g0695800	NA efflux transporter	↑(root)↓(shoot)	Nozoye et al. 2011
OsYSL2	Os02g0649900	Ferrous Fe/Mn-NA transporter	↑	Ishimaru et al. 2010
OsHMA2	Os06g0700700	Cd/Zn transporter	↑	Yoneyama et al. 2015
OsLCT1	Os06g0579200	Cd efflux transporter	—	Uraguchi et al. 2011
Transporters for cellular Fe/Cd sequestration				
OsVIT1	Os04g0463400	Fe import into vacuole	→	Pich et al. 2001; Zhang et al. 2012
OsVIT2	Os09g0396900	Fe import into vacuole	↓	Pich et al. 2001; Zhang et al. 2012
OsHMA3	Os07g0232900	Cd import into vacuole	—	Takahashi et al. 2012a
OsABCG43	Os07g0522500	Cd import into vacuole	—	Oda et al. 2011

[a]Arrows indicate rice genes expressional response: "↑", upregulated; "↓", downregulated; "→", no significant change; "—", not determined

Arabidopsis thaliana, AtHMA3, similar as OsHMA3, is responsible for sequestration of Cd into vacuoles (Miyadate et al. 2011). Meanwhile, an allele of *OsHMA3* was discovered to fail to transport Cd into vacuole in Cd-high-accumulating cultivars such as some *indica* cultivars. Owing to non-function of OsHMA3, Cd is accelerated to distribute within rice, leading to high accumulation. These cultivars presented high Cd accumulation in the shoots and grains (Miyadate et al. 2011).

Translocation of Fe and Cd from roots to shoots

Following uptake by roots, Fe and Cd are transported to shoots via xylem and phloem, where exist a large amount of vascular bundles (Yoneyama et al. 2015). This radial transport system includes symplasmic and apoplasmic pathways, but the former pathway is predominantly utilized as a result of impediment by Casparian strips occuring in apoplasmic pathway (Enstone et al. 2002). After Fe(II)-NA formation in the cytosol, Fe(II)-NA is transported to xylem and exchanges NA with citrate, transforming to Fe(III)-citrate preferentially (Yokosho et al. 2009; Ariga et al. 2014). 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 (Yoneyama et al. 2015). The translocation of citrate from root pericycle cells to xylem is mediated by ferric reductase defective1-like transporter (OsFRDL1) (Yokosho et al. 2009) (Fig. 1d). *OsFRDL1* is constitutively expressed in root pericycle cells and transporter OsFRDL1 is specifically required for Fe translocation (Yokosho et al. 2009).

Phloem loading is the upcoming step. Transporter OsYSL2 plays a part 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 (Ishimaru et al. 2010) (Table 1). OsYSL2 knock-down rice lines accumulated less Fe and Mn in shoots and seeds (Kobayashi et al. 2010). With regard to mechanism for efflux of NA into xylem in specific way, Nozoye et al. (2011) proposed that the NA efflux transporters (ENA1/2) are responsible for the effux of NA into xylem or intracellular compartments in order to redistribute Fe (Fig. 1d). Under Fe deficiency, both *OsYSL2* and *ENA1* are strongly induced (Ishimaru et al. 2010; Ogo et al. 2014). In addition to transporter OsYSL2, OsYSL15 is considered to transport Fe(III)-DMA for phloem trafficking and expressed in the phloem companion cells (kobayashi et al. 2010; kakei et al. 2012; Kobayashi et al. 2014) (Fig. 1d). Thereafter, Fe is delivered to grain via phloem in forms of Fe(III)-DMA or binds to some citrate and proteins (Yoneyama et al. 2015).

As for Cd translocation, once Cd enters 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. Such xylem loading occurs in root pericycle cells with OsHMA2 (Takahashi et al. 2012b; Yamagi et al. 2013; Yoneyama et al. 2015) (Fig. 1c). Moreover, OsHMA2 is also involved in xylem-to-phloem transfer (Yoneyama et al. 2015). In the phloem, Cd primarily bounds to specific proteins and slightly to thiol-compouds (White and Broadley 2011). 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 (Fujimaki et al. 2010). In rice nodes, the diffuse vascular bundles (DVBs) that encircle the enlarged elliptical vascular bundles (EVBs) are connected to the panicle (Yamaguchi et al. 2012). A study demonstrated that Cd was predominantly transported towards the panicle instead of other tissues at the panicle-initiation stage through the nodes and ultimately reached grain by prositron-emitting [107]Cd tracer imaging system (PETIS) (Fujimaki et al. 2010). Node I, the uppermost node, is connected to both flag leaf and panicle. The large vascular bundles (LVBs) of flag leaf are linked to the EVBs. Metals, such as Cd, that are not transported to the panicle can be shifted to flag leaf (Uraguchi et al. 2011). Yamaguchi et al. (2012) found that Cd concentration was higher in the node I than in blade, culm and panicle due to the accumulation of Cd. Furthermore, A low-affinity cation transporter (OsLCT1), which is highly expressed in the node I, participates in Cd transport to grain (Uraguchi et al. 2011) (Fig. 1c). OsLCT1 is identified as a plasma membrane-localized transporter by subcellular localization of OsLCT1-sGFP (Uraguchi et al. 2011). Suppression of *OsLCT1* exppression can efficiently decrease grain Cd levels (Uraguchi et al. 2011).

Therefore, Node is deemed to be an important "transportation junction" responsible for Cd distribution. Shoot base contains the lower packed nodes with numerous vascular bundles, designated as the "traffic control centre", and where Cd can be separated into the tillers, accumulating in each node (Fujimaki et al. 2010).

Role of cellular sequestration in mitigating Cd toxicity

As stated in the introduction section above, Plants have developed numerous resistance mechanisms against Cd toxicity, "avoidance" and "tolerance" included. "Avoidance" is first employed and serves as a basic mechanism to relieve Cd toxicity. However, "tolerance", which makes plants survive in the presence of Cd stress, plays a major role in mitigating Cd toxicity. In the "tolerance" mechanisms, deposition of Cd in the cell wall is a first barrier to restrict Cd movement by combining with composition of cell wall (Carrier et al. 2003; Choppala et al. 2014) (Table 2). Xiong et al. (2009) first proved that exogenous NO could be involved in regulation of root cell wall composition to alleviate Cd toxicity. Enhanced

Table 2 Comparison on mechanisms of Fe/Cd detoxicfication in rice

Mechanisms	Fe	Cd	References
Cellular sequestration			
Storage sites	Vacuole in the flag leaves and sheaths	Vacuole in the root cells and leaves	Choppala et al. 2014; Pich et al. 2001;
Chemical forms	Fe(II)-NA	Cd-PC	Takahashi et al. 2012a;
Mediated transporters	OsVIT1/2	OsHMA3	Zhang et al. 2012
Location of transporters	Tonoplast	Tonoplast	
Combination of Fe/Cd and organics			
Storage sites	Chloroplast or embryo in the leaves and seeds	Cell wall in the roots and leaves	Ravet et al. 2009; Xiong et al. 2009
Chemical forms	Fe-Ferritin	Cd-Pectin and Cd-Hemicellulose	

pectin and hemicellulose contents (Table 2) induced by exogenous NO in root cell wall increased Cd deposition in cell wall and decreased Cd distribution within rice. Once entering into cell, PC that acts as a chelator can make complexes with Cd, forming Cd-PC (Table 2). Basic structure of PC, consisting of glutamate, cysteine and glycine has been identified (Rauser 1995), and glutathione (GSH) is a key intermediate for the biosynthesis of PC and catalyzed by PC synthase (PCS) (Rauser 1995; Cobbett 2000). PCS can be activated in the presence of Cd (Cobbett 2000). As for rice, in the root cells, Cd-PC complexes are stored in the vacuoles mediated by tonoplast-localized transporter OsHMA3 (Tables 1 and 2), followed by dissociation in the vacuoles due to acidic environment (Johanning and Strasdeit 1998; Takahashi et al. 2012a, b; Choppala et al. 2014). Dissociated PC can be recycled in the next round (Johanning and Strasdeit 1998).

According to this cellular mechanism, subsequent movement of Cd through the root symplasm to the xylem can be limited (Nocito et al. 2011). Some reports pointed that OsHMA3 is expressed in shoots besides roots, which implies the key factor of OsHMA3 in determining root-to-shoot transfer of Cd and regulation of Cd distribution within rice. Particularly, cellular sequestration mediated by OsHMA3 in root cells is a rate-limiting step (Ueno et al. 2010). Furthermore, an ABC-type transporter OsABCG43 is also considered to be a probable candidate for Cd tolerance in rice (Oda et al. 2011; Uraguchi and Fujiwara 2012). OsABCG43 is likely to sequester Cd at the subcellular level, as well as vacuolar sequestration mediated by OsHMA3 (Oda et al. 2011) (Table 1). Nevertheless, whether there are other mechanisms mediated by OsABCG43 is far from clear.

Mechanisms for maintaining Fe homeostasis
Despite enhancing Fe is primary concern to agricultural production, over-accumulated Fe can cause cellular damage since Fe is highly reactive (Conte and Walker, 2011). Fe(II) is considered to be a source of reactive oxygen species (ROS), which results in oxidative damage (Curie et al. 2009). Thus, maintaining Fe homeostasis is crucial to plant growth. Plant has evolved Fe homeostatic mechanisms that regulate Fe acquisition. Once taken up into cells, Fe is subjected to strict control to avoid cellular damage. Ferritin, a Fe storage protein, can combine with Fe atoms in bioavailable and non-toxic form for distribution within plants and protect against Fe-mediated oxidative stress (Liu et al. 2003; Ravet et al. 2009) (Table 2). Besides ferritin, nitric oxide (NO) is a promising candidate for serving as a scavenger of ROS, and the reduction of nitrite by nitrite reductase (NR) is a major pathway to generate NO (Crawford 2006). Moreover, vacuolar sequestration is another significant mechanism in controlling Fe homeostasis. Vacuole functions as buffering pool in conditions of Fe toxicity through the interaction between tonoplast-localized transporters and Fe chelators (Table 2). As mentioned from the above, NA is also involved in intracellular movements and acts as a strong chelator of Fe (Table 2). Excess Fe, as well as Cd compartmentalization, can be chelated by NA and sequestrated in the vacuole (Pich et al. 2001). In rice, *OsVIT1* and *OsVIT2* that encode vacuolar transporters are highly expressed in flag leaf and sheath, respectively, transporting excess Fe into vacuole (Zhang et al. 2012) (Tables 1 and 2).

Effects of fertilizers on Fe and Cd accumulation discrepancy in rice
Enhancing Fe concentration in grains through water-fertilizer management is a kind of agronomic biofortification though the strategy plays a role in a short term (Slamet-Loedin et al. 2015). Nitrogen (N) is an essential macronutrient for plants (Sarwar et al. 2010). N application promotes YSL protein synthesis and nitrogenous compounds formation, such as NA and DMA, both of which participate in Fe transport in rice (Slamet-Loedin et al. 2015) (Table 3). Moreover, increased N application results in more biomass production and reduces Cd toxicity to some extent due to dilution effect

Table 3 Positive and negative effects caused by fertilizer types on Fe/Cd accumulation in rice

Fertilizer types	Positive effects	Negative effects	References
Nitrogen (NH_4^+/NO_3^-)	Increased YSL protein synthesis and nitrogenous compounds formation for Fe transport	Decreased soil pH and membrane depolarization by NH_4^+ application	Zaccheo et al. 2006; Wangstrand et al. 2007; Xie et al. 2009; Sarwar et al. 2010; Slamet-Loedin et al. 2015; Yang et al. 2016b
	Increased soluble protein content reduce mobility of Cd	Up-regulated expression of Fe/Cd co-transporters by excess NO_3^- application	
	Higher antioxidase activity by NH_4^+ application reduce Cd toxicity		
	High soil pH and membrane polarization by NO_3^- application produce Cd detoxcification		
Phosphorus	Insoluble Cd formation in soil	Limited source of P fertilizer	Cordell et al. 2009; Wang et al. 2009; Sarwar et al. 2010
	GSH biosynthesis participation	Decreased soil pH enhance solubility of Cd	
	Increased antioxidase activity by P application		
Iron	Compete with Cd for the same binding site under anaerobic conditions	Increased Cd concentration by some Fe^{2+} fertilizers (e.g. $FeSO_4$) application	Sharma et al. 2004; Shao et al. 2008; Liu et al. 2008; Rizwan et al. 2016
	Alleviate oxidative stress caused by Cd		
	Iron plague formation		
Zinc	Compete with Cd for the same transporters	Simultaneous Zn/Cd absorption by root cells	Smilde et al. 1992; Aravind et al. 2009; Sarwar et al. 2010; Fahad et al. 2015; Rizwan et al. 2016
	Alleviate oxidative stress caused by Cd	Enhanced Cd concentration caused by high level of Zn	
Silicon	Increased soil pH reduce mobility of Cd		Sarwar et al. 2010; Wang et al. 2015; Rizwan et al. 2016
	Si-Cd complexes formation		
	Enhanced antioxidase activity		
	Enhanced Fe level		
Sulfur	Insoluble CdS formation reduce mobility of Cd	Increased soil pH enhance Cd concentration and mobility	Hassan et al. 2005; Rehman et al. 2015
	GSH biosynthesis participation		
	Iron plague formation		

(Sarwar et al. 2010). Such effect is mainly caused by increased soluble protein content in crops that can transform mobile Cd to immobile form by binding (Sarwar et al. 2010) (Table 3). Different N fertilizer forms also have relationships with Fe/Cd uptake and accumulation in both roots and shoots (Mitchell et al. 2000). Ammonium (NH_4^+) and nitrate (NO_3^-) are primary N fertilizer forms for rice absorption and assimilation (Jalloh et al. 2009). In the paddy soil, NH_4^+-containing fertilizer is predominantly employed (Araki et al. 2015). Higher antioxidase activity exposure to NH_4^+ treatment is considered as protective mechanism against Cd stress (Rizwan et al. 2016) (Table 3). Although the preferential application of NH_4^+ over NO_3^- as a nitrogen source for rice, many reports proved that combination of NH_4^+ and NO_3^- is better for rice growth (Sarwar et al. 2010; Araki et al. 2015).

Cd in acidic soil is ionized as Cd^+ (Khaokaew et al. 2011), which can promote mobilization of Cd (Sarwar et al. 2010). Rhizosphere acidification caused by NH_4^+-containing fertilizer derives from proton excretion by root

cells, exchanging with NH_4^+ and leading to low pH in soil (Zaccheo et al. 2006). In low pH soil, Cd is mobile to move towards root system and translocates within plants, resulting in Cd accumulation (Table 3). 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 mechanisms reduces Cd uptake in a certain way (Zaccheo et al. 2006; Sarwar et al. 2010) (Table 3). Consequently, NH_4^+-containing fertilizer is considered to contribute to enhance Cd uptake (Sarwar et al. 2010). Compared with NH_4^+-containingfertilizer, NO_3^--containing fertilizer causes simultaneous NO_3^- and proton absorption by root cells, leading to high pH (Eriksson, 1990) and cell membrane polarization caused by nitrate can produce Cd detoxification mechanism (Sarwar et al. 2010) (Table 3). Nevertheless, Xie et al. (2009) found that plants supplied with NO_3^- accumulated more Cd than NH_4^+ treatment by *Thlaspi caerulesscens* 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. (2016b) found that rice fed with excess NO_3^- not only enhanced Fe uptake, but also increased Cd uptake by up-regulating the expression of *OsIRT1*. It deserves further study that N fertilizer might enhance Cd uptake and accumulation resulting from up-regulated expression of common Cd/Fe transporter genes (Table 3). Wangstrand et al. (2007) once proposed that application of N fertilizer is 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. Therefore, the application of N fertilizer on rice can be manipulated to decrease Cd content and enhance Fe content. Meanwhile, selection of proper N fertilizer forms according to different growth stages is still hot research field.

Besides application of N fertilizer, other mineral fertilizers also contribute to rice growth as well. Phosphorus (P) is another important plant nutrient and applied to plant as fertilizer. In contrast to N, P is a non-renewable natural resource and there is growing concern about limited source of P fertilizer (Cordell et al. 2009). Most of P are derived from rock phosphate containing Cd and hence result in Cd contamination in soil (Lambert et al. 2007; Sarwar et al. 2010). Sarwar et al. (2010) mentioned that mono-ammonium-phosphate (MAP) could enhance Cd uptake due to increased solubility of Cd by lowering soil pH (Table 3). However, P fertilizer also reduce solubility of Cd by insoluble Cd formation, such as $Cd(OH)_2$ or $Cd_3(PO_4)_2$ (Bolan et al. 2003) (Table 3). Furthermore, crops have developed intrinsic mechanisms against Cd stress caused by rock phosphate containing Cd (Sarwar et al. 2010; Slamet-Loedin et al. 2015). Slamet-Loedin et al. (2015) mentioned that minor effect of P fertilizer-related Cd uptake on rice. P is involved in glutathione (GSH) biosynthesis (Table 3), which is precursor of PC. Recently, Yang et al. (2016a) proposed that P deprivation decreases Cd uptake by inhibiting biomass accumulation and reducing PCs synthesis. As elucidated above, in rice, PC can form complexes with Cd after Cd is transported into vacuole, alleviating Cd toxicity efficiently (May et al. 1998; Sarwar et al. 2010). On the other hand, an increase in antioxidase activity by the application of P plays an indispensable role in alleviating oxidative stress caused by Cd toxicity (Wang et al. 2009) (Table 3). In summary, as well as N application, appropriate P application is necessary for rice growth.

Application of Fe fertilizer is direct and effective method for enhancing Fe content while reducing Cd toxicity to some extent. Under anaerobic conditions, such as flooded status, Fe^{2+} is dominant chemical form in soil (Sarwar et al. 2010). Owing to similar chemical form, Fe^{2+} compete with Cd for the same binding site and transport systems on the surface of root cells (Table 3),

reducing Cd uptake in a certain way accordingly (Sarwar et al. 2010). Nevertheless, different types of Fe^{2+} fertilizer may have discrepant effects on Cd accumulation. Shao et al. (2008) showed that application of $FeSO_4$ remarkably increased Cd concentrations in roots and shoots of rice (Table 3). Thus, selection of appropriate Fe type is conducive to rice growth. Furthermore, Fe is important co-factor of antioxidase, which can provide protective mechanisms against oxidative stress (Sharma et al. 2004) (Table 3). A peculiar mechanism against Cd stress by application of Fe fertilizer is iron plague (IP) formation (Rizwan et al. 2016) (Table 3). This 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 (Liu et al. 2008).

Zinc is an essential micronutrient for crops growth. As a result of similar physical and chemical characteristics (Rizwan et al. 2016), the interactions between Zn and Cd, synergistic and antagonistic effect (Fahad et al. 2015; Rizwan et al. 2016), have been an issue to be solved for the field of agricultural science. In antagonistic way, Zn will compete with Cd for the same membrane transporters (Table 3), restricting Cd uptake by root cells. In synergistic way, both of Zn and Cd are absorbed by root cells, increasing Cd accumulation in rice (Sarwar et al. 2010) (Table 3). Xue and Harrison (1991) discovered that higher level of Zn fertilizer application causes enhanced Cd concentration in lettuce leaves. Smilde et al. (1992) also identified that increased Zn concentration in soil leaded to high Cd concentration in leafy vegetables (Table 3). Hence, controlling Zn fertilizer application at an optimum level may reduce Cd concentration caused by synergistic effect. In addition, Zn application is involved in alleviating oxidative stress (Table 3) caused by Cd and has protective mechanisms against reactive oxygen species (ROS) that result in cell membrane damage (Aravind et al. 2009).

Silicon (Si) is thought to be an enhancer for crops growth though it is not an essential element (Rizwan et al. 2016). Wang et al. (2015) found that Si application could enhance Fe level in rice (Table 3). Si application can reduce mobility of Cd due to increased pH in soil (Sarwar et al. 2010) (Table 3). Complexes formation of Si with Cd is another mechanism for alleviating Cd toxicity in rice (Rizwan et al. 2016) (Table 3). Moreover, Si application can also enhance antioxidase activity (Sarwar et al. 2010) (Table 3).

Sulfur is a significant component of many co-factor of enzymes (Table 3), having an indispensable effect on crops growth (Sarwar et al. 2010). Application of S fertilizer may decrease Cd toxicity by insoluble CdS formation (Table 3), by which reduces mobility of Cd in soil (Hassan et al. 2005). As well as effect of P fertilizer application, S also participates in GSH biosynthesis (Table 3),

forming Cd-PC complexes and reducing Cd toxicity by compartmentalization of Cd into vacuole (Cobbett 2000; Sarwar et al. 2010; Rizwan et al. 2016). However, Rehman et al. (2015) suggested that application of S fertilizer might enhance Cd concentration in rice grains by lowering soil pH and increase mobility of Cd (Table 3), which is negative effect of S fertilizer application. Therefore, Rizwan et al. (2016) proposed that combination of different mineral fertilizers contributed to decrease Cd uptake by rice compared with respective application. For instance, Wei et al. (2012) found that foliar application of combined Fe and Zn fertilizers might increase Fe and Zn content, decreasing Cd content in rice grains.

Approaches for reducing grain Cd accumulation and enhancing Fe content

Soil remediation methods are traditionally applied to reduce Cd toxicity to some extent, including soil removal, replacement, inversion and flooded condition before and after heading (Arao et al. 2009; Uraguchi and Fujiwara 2012). In addition, establishment of "low-Cd-rice" based on genetic findings is considered to be an effective approach to reduce Cd accumulation. There is genotypic variation in the Cd levels of grains in different rice cultivars. Cd accumulation in shoots and grains are greater in *indica* rice cultivars than in *japonica* cultivars (Ishikawa et al. 2005; Takahashi et al. 2011). Quantitative trait locus (QTL) analysis is a useful approach to identify responsible genes for the respective transport processes, such as various transporters (Ishikawa et al. 2010). QTL for Cd concentration in Anjana Dhan (*indica* rice cultivar) is identified on chromosome 7, responsive gene for which is *OsHMA3* (Ishikawa et al. 2010; Ueno et al. 2010; Takahashi et al. 2011). Amino acid at position 80 plays a key role in the function of OsHMA3 and mutation of this amino acid in Anjana Dhan makes Cd fail to be sequestered into vacuoles in root cells, accelerating translocation of Cd from roots to shoots (Ueno et al. 2010; Takahashi et al. 2011). Abe et al. (2011) 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. Regulation of genes for Cd transporters can also effectively reduce Cd accumulation in rice (Ueno et al. 2010). Suppression of *OsLCT1* expression can decrease grain Cd accumulation by RNAi without influencing nutrient accumulation. On the contrary, Fe content in the brown rice is remarkably higher (Uraguchi et al. 2011), suggesting that RNAi-mediated *OsLCT1* suppression in rice is a promising approach to establish "high Fe but low-Cd-rice". Furthermore, a novel rice gene *low cadmium* (*LCD*) is related to Cd tolerance (Uraguchi and Fujiwara, 2012). This T-DNA-mediated *OsLCD* knockout mutant showed reduced

grain Cd accumulation by insertion into the first intron of *OsLCD*, having no negative effects on grain yield (Shimo et al. 2011). The authors indicated that the *lcd* mutant might be a probable mutant line for further research.

Improving bioavailability of Fe is the main goal for breeding Fe-rich rice. A variety of approaches have been utilized to enhance Fe content in grains. Goto et al. (1999) demonstrated that high level of Fe in rice endosperm could be acquired by overexpression of ferritin. Combination of up-regulated expression of ferritin with overproduction of NA can significantly enhance Fe content (Wirth et al. 2009). Zheng et al. (2010) indicated that biofortifying rice with NA could efficiently enhance Fe bioavailability by over-expression *OsNAS1* in rice endosperm, suggesting that NA plays a great potential role in enhancing Fe bioavailability. In addition, manipulation of specific transporters involved in Fe uptake and translocation is considered to be another promising approach for enhancing Fe content. Ishimaru et al. (2010) 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.

In recent years, studies on rice screened for Fe-rich but Cd-free cultivars have been an important issue to agricultural field. Olive et al. (2014) bred an over-accumulated ferritin cultivars with rice mega-variety IR64 that serve as background. Compared with IR64 wild type, Fe content was increased in grains by introducing ferritin into endosperm. Such a ferritin-bioengineered diet is considered to be effective for mammal assimilation of Fe nutrition (Murray-Kolb et al. 2002; Olive et al. 2014). Moreover, Booyaves et al. (2016) expressed Arabibopsis IRT1(*AtIRT1*) in high-iron NFP rice lines, which expressed *NICOTIANAMINE SYNTHASE* (*AtNAS1*) and *FERRITIN*, suggesting that coordinated expression of *AtIRT1*, *AtNAS1* and *PvFERRITIN* enhanced Fe content in both unpolished and polished grains. Thus, combined expression of genes encoding Fe transporters or Fe storage proteins are promising approach for Fe biofortification. With regard to breeding low Cd cultivars, Ishikawa et al. (2012) 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 (Ishikawa et al. 2012). 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 non-function of OsNRAMP5 and decreased Cd accumulation in grains (Ishikawa et al. 2012). Similar results were observed in *lcd-kmt2* and *lcd-kmt3* due to a single-base pair deletion and a large deletion in *OsNRAMP5*, respectively (Ishikawa et al. 2012). Meanwhile, Ishikawa et al. (2012) 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* (Ishikawa et al. 2012). In addition, Abe et al. (2013) developed a novel population composed of 46 chromosome segment substitution lines (CSSLs), in which LAC23 served as donor segments and were substituted into background, Koshihikari. LAC23 could result in lower grain-to-straw ratio than Koshihikari (Abe et al. 2013). Therefore, Cultivars containing LAC23 performed low Cd content in grains (Abe et al. 2013). QTL mapping detected a major QTL, *qlGCd3*, and LAC23 allele at *qlGCd3* were identified to be related with reduced Cd content in grains (Abe et al. 2013). This result showed that low-Cd trait of LAC23 is promising for breeding low-Cd rice cultivars.

Conclusions and perspectives

Fe is essential nutrient for rice growth and humans consuming rice as their staple food, whereas Cd is non-essential and toxic. Rice grains contain both Fe and Cd. The latter may cause damage to human bodies if accumulating at high levels. Basic transport processes are as follows: During the vegetative stage, Fe and Cd are absorbed by specific root transporters and then transport to xylem, delivering to aerial parts via xylem-to-phloem transfer system. Compared with Cd of which such transfer system mainly operating at nodes, Fe is preferentially allocated to leaves through xylem. At grain-filling, grain Fe and Cd are derived from phloem. Particularly, grain Fe is largely acquired from leaves by remobilization. Due to limited source of Fe in soil and Cd toxicity, rice has evolved mechanisms against Fe deficiency and Cd stress. Rice can secrete DMA to chelate insoluble Fe^{3+} by strategy II. In response to Cd toxicity, compartmentalization of Cd into vacuole is thought to be effective tolerance mechanism for reducing Cd translocation to grains. However, relying on rice self-mechanisms are not enough to tackle issues of Fe deficiency and Cd toxicity. Besides traditional soil remediation and fertilizers management, breeding high Fe but low Cd cultivars through genetic methods are considered as promising approaches based on understanding of Fe/Cd transport and accumulation processes at cellular level. QTL analysis, regulation and manipulation of genes involved in Fe/Cd accumulation are generally utilized to produce novel cultivars.

Despite these advances in enhancing Fe in grains, limited Fe in edible parts due to combining with phytic acid and inevitable loss during polishing process restricts Fe nutritional assimilation for human bodies. In addition, some low-Cd cultivars are reported to enhance other toxic metals, such as Arsenic, though reduce Cd content to some extent. Thus, further investigation into these issues will be conducive to develop Fe-fortified cultivars with increased assimilation and low-Cd cultivars without other toxic metals accumulation.

Authors' contributions
LG wrote the manuscript with critical revision by JX, HFL and LXT. JDC participated in the data analysis. RJC and HBL participated in collecting the data of the related researches. All of the authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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