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Review:

Breeding for low cadmium accumulation cereals*

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Abstract: Cadmium (Cd) is an element that is nonessential and extremely toxic to both plants and human beings. Soil contaminated with Cd has adverse impacts on crop yields and threatens human health via the food chain. Cultivation of low-Cd cultivars has been of particular interest and is one of the most cost-effective and promising approaches to minimize human dietary intake of Cd. Low-Cd crop cultivars should meet particular criteria, including acceptable yield and quality, and their edible parts should have Cd concentrations below maximum permissible concentrations for safe consumption, even when grown in Cd-contaminated soil. Several low-Cd cereal cultivars and genotypes have been developed worldwide through cultivar screening and conventional breeding. Molecular markers are powerful in facilitating the selection of low-Cd cereal cultivars. Modern molecular breeding technologies may have great potential in breeding programs for the development of low-Cd cultivars, especially when coupled with conventional breeding. In this review, we provide a synthesis of low-Cd cereal breeding.

Key words: Cereals; Low Cd accumulation; Gene/quantitative trait locus (QTL) mapping; Breeding
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1 Introduction

Soil cadmium (Cd) contamination poses a serious problem for safe food production and has become a potential agricultural and environmental hazard worldwide. Cd contamination may be caused by industrial emissions or applications of Cd-containing sewage sludge, phosphate fertilizers, or municipal waste (Čásová et al., 2009; Roberts, 2014; Liu et al., 2015; Yousaf et al., 2016). Particularly, soils derived from marine shales or contaminated by mine waste are likely to have a high Cd content (Arunakumara et al., 2013). Cd has high soil-to-plant mobility and can easily accumulate in plant tissues (Song et al., 2015), causing reduced crop yields and threatening human health via the food chain (Clemens et al., 2013; Sun et al., 2013). In humans, Cd accumulates mainly in the kidneys and has a biological half-life of about 20 years (Clemens et al., 2013; Aziz et al., 2015). This very slow elimination of accumulated Cd can lead to a variety of serious health issues including anemia, cancer, cardiovascular disease, and renal tubular damage (Satarug et al., 2003, 2009). Extreme cases of chronic Cd toxicity can result in osteomalacia and bone fractures, such as the "Itai-Itai" disease that occurred in Japan during the 1950s and 1960s (Huang et al., 2009). As a result of these and other illnesses, Cd contamination in soils has been of increasing concern for decades in many industrialized and developing countries (Arthur et al., 2000; Wu et al., 2004; Cao et al., 2014a).

Soil Cd contamination poses considerable risks to human health via the consumption of foods containing high Cd concentrations, especially staple foods such as cereals (Stolt et al., 2003). For example, rice

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ORCID: Fei-bo WU, https://orcid.org/0000-0001-8797-3398; Qin CHEN, https://orcid.org/0000-0002-3964-321X has been a major dietary source of Cd for the Japanese population (Tsukahara et al., 2003), contributing about 30% of the total dietary Cd intake in Japan during the 1990s (Ikeda et al., 1999). Dabeka et al. (1987) found that the Cd content of cereals was the highest among ten classified food groups in Canada. Grain Cd concentrations as high as 1–2 mg/kg dry weight (DW) have been recorded in rice grown in some areas of central-southern and southwestern China (Du et al., 2013; Chen et al., 2018). Those levels are much higher than the maximum permissible concentration (MPC) for cereal grains as defined by the Chinese National Standard (NHFPC, 2017) and the Codex Alimentarius Commission (CAC, 2019). According to national average estimates of exposure, cereals contribute about 32% of the total Cd intake by the Chinese population (WHO, 2011). Therefore, it is imperative to reduce Cd accumulation in cereal grains so as to control Cd intake by human beings.

The development and planting of low-Cd cultivars is a cost-effective and environmentally friendly approach to minimize soil–plant transfer of Cd and produce safe food for slightly or moderately contaminated soils. For over a decade, breeding programs have been initiated to select for low-Cd crop cultivars (Oliver et al., 1995). Many studies have been conducted to screen and develop low-Cd cultivars in a variety of crop species, including rice, barley, maize, and wheat (Oliver et al., 1995; Wu and Zhang, 2002; Yu et al., 2006; Chen et al., 2007a, 2007b; Wu et al., 2007; Grant et al., 2008; Zeng et al., 2008). In this review, we focus on recent advances in selecting low-Cd cereal crops, including: (1) genes related to Cd accumulation; (2) quantitative trait loci (QTLs) associated with Cd accumulation; and (3) breeding low-Cd cultivars.

2 Genes associated with Cd accumulation in cereal crops

For developing low-Cd crop cultivars, it is important to identify genes related to Cd accumulation and to understand the mechanisms of Cd uptake and accumulation in plants. In recent years, a number of genes related to Cd transport in cereals have been identified (Table 1), and progress has been made in understanding the mechanisms of Cd uptake and transport (Chen JG et al., 2019). Cd is not essential for plant growth, but its transport in plants is mediated by the transporters for essential elements such as Zn, Mn, Ca, and Fe (Uraguchi and Fujiwara, 2013; Clemens and Ma, 2016). Some genes related to chelation have also been found to play roles in plant Cd homeostasis.

2.1 Transporter-related genes

In plants, natural resistance-associated macrophage proteins (NRAMPs) are a family of metal transporters that are integral components of membranes and play important roles in metal uptake, translocation, and intracellular transport (Nevo and Nelson, 2006; Sasaki et al., 2012). *OsNramp5*, a Mn transporter located at the plasma membrane of root cells, was found to be a major transporter of Cd uptake in rice roots exposed to Cd-containing soil medium. Knockout or knockdown of *OsNramp5* resulted in more than a 90% reduction in Cd uptake (Sasaki et al., 2012; Yang M et al., 2014), thereby drastically reducing the Cd content of shoots and grains (Ishimaru et al., 2012; Tang et al., 2017). Transfer DNA (T-DNA) insertion mutants of *OsNramp5* led to large yield reductions of about 11% compared with wild-type plants (Sasaki et al., 2012), while *OsNramp5* knockout lines developed by ion-beam irradiation (Ishikawa et al., 2012) or by the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system (Tang et al., 2017) did not affect grain yield. Orthologues of *Nramp5* in other cereal crops have a similar function. *TpNramp5* in dwarf Polish wheat functions as a metal transporter for Cd, Mn, and Co. When this gene was expressed in *Arabidopsis*, it significantly increased Cd, Co, and Mn concentrations in roots, shoots, or the whole plant (Peng et al., 2018b). Similarly, knockdown of the orthologue in barley, *HvNramp5*, which is preferentially expressed in the outer root cell layers and root tips, resulted in significant reductions of Mn and Cd concentrations in both roots and shoots (Wu et al., 2016), but its relative contribution appears to be smaller than that of *OsNramp5* in rice. Comparison of the expression levels and transport activities of three *Nramp5* orthologues from rice, wheat, and maize, showed that *OsNramp5* in rice was expressed at higher levels, resulting in higher Cd transport activity than *Nramp5* in wheat or maize (Sui et al., 2018). Other NRAMP members in cereal crops also show Cd

Table 1 List of reported genes of cereal crops related to Cd homeostasis

Gene	Gene name	Probable function	Reference
Oryza sativa[1]			
OsNramp1	Natural resistance-associated macrophage protein	Cd and Fe transporters	Takahashi et al., 2011a, 2011b
OsNramp5		Cd, Mn, and Fe transporters	Ishikawa et al., 2012; Sasaki et al., 2012; Yang M et al., 2014; Tang et al., 2017
OsIRT1	Zinc- and iron-regulated transporter	Cd and Fe transporters	Nakanishi et al., 2006; Lee and An, 2009
OsIRT2			Nakanishi et al., 2006
OsZIP1		Cd and Zn transporters	Bashir et al., 2012; Ramegowda et al., 2013; Liu XS et al., 2019
OsZIP7		Cd and Zn accumulation	Ricachenevsky et al., 2018; Tan et al., 2019
OsHMA2	P-type heavy metal ATPase	Cd and Zn translocation	Satoh-Nagasawa et al., 2012; Takahashi et al., 2012; Yamaji et al., 2013
OsHMA3		Sequestration of Cd in root	Ueno et al., 2010; Miyadate et al., 2011; Sasaki et al., 2014; Yan et al., 2016; Shao et al., 2018; Lu et al., 2019
OsHMA9		Cd efflux	Lee et al., 2007
OsLCD	Low cadmium	Cd tolerance and accumulation	Shimo et al., 2011
OsCd1	Major facilitator superfamily	Cd uptake	Yan et al., 2019
OsLCT1	Low affinity cation transporter	Cd transporter in phloem	Uraguchi et al., 2011, 2014
OsCCX2	Cation/Ca^{2+} exchanger 2	Cd tolerance and translocation	Hao et al., 2018
OsMTP1	Metal tolerance protein gene	Cd translocation	Yuan et al., 2012
OsPCS2	Plant chelatase synthase 2	Cd tolerance and accumulation	Das et al., 2017
OsPCS1	Plant chelatase synthase 1	Cd tolerance and accumulation	Uraguchi et al., 2017
OsMTI-1b	Metallothionein-like protein 1b	Cd tolerance and accumulation	Ansarypour and Shahpiri, 2017
CAL1	Defensin-like protein	Cd accumulation in leaf	Luo et al., 2018
Triticum aestivum			
TaHMA2	P-type heavy metal ATPase	Cd and Zn transporters	Tan et al., 2013
HMA3-B1		Cd accumulation in grains	Wiebe et al., 2010
Triticum polonicum			
TpNRAMP3	Natural resistance-associated macrophage protein	Cd, Mn, and Co transporters	Peng et al., 2018a
TpNRAMP5		Cd, Mn, and Co transporters	Peng et al., 2018b
Zea mays			
GRMZM2G 175576	Homologous to rice OsHMA2 and OsHMA3	Cd accumulation	Zhao XW et al., 2018
Hordeum vulgare			
HvIRT1	Zinc-and iron-regulated transporter	Fe, Mn, Zn, and Cd transporters	Pedas et al., 2008
HvZIP3		Mn, Zn, and Cd accumulation	Sun et al., 2015
HvZIP8		Mn, Zn, and Cd accumulation	Sun et al., 2015
HvHMA2	P-type heavy metal ATPase	Zn, Fe, and Cd transporters	Barabasz et al., 2013
HvHMA3		Cd accumulation	Wu et al., 2015
HvNramp5	Natural resistance-associated macrophage protein	Mn and Cd accumulation in roots and shoots	Wu et al., 2016

[1] List of reported genes in rice (*Oryza sativa*) related to Cd homeostasis was reviewed by Chen JG et al. (2019)

uptake and transfer activities. The plasma membrane-localized iron transporter, OsNRAMP1, may assist in loading Cd into the xylem. Thus, over-expression of *OsNRAMP1* in rice increased Cd accumulation in leaves while reducing Cd levels in roots (Takahashi et al., 2011a). *TpNramp3* from dwarf Polish wheat has a similar function to *TpNramp5*, and is also a transporter for Cd, Mn, and Co. Heterologous expression of *TpNramp3* in *Arabidopsis* resulted in increased Cd, Co, and Mn concentrations in roots, shoots, and whole plants, but did not affect their translocation from roots to shoots (Peng et al., 2018a).

In addition to the NRAMP family, several members of the zinc/iron-regulated transporter-like proteins (ZIPs) family, the primary zinc and iron transporters in plants, are also involved in Cd uptake. Some Fe transporters have been found to participate in plant Cd uptake. Nakanishi et al. (2006) found that *OsIRT1* and *OsIRT2* play important roles in Cd uptake. Over-expression of *OsIRT1* resulted in increased Cd accumulation (Lee and An, 2009). In *Arabidopsis*, over-expression of *AtIRT1* and *AtIRT2* also caused an increase in Cd accumulation (Connolly et al., 2002; Vert et al., 2009). Similarly, *HvIRT1*, a plasma membrane-localized transporter for Fe, Mn, and Zn, also exhibits Cd transport activity when expressed in yeast (Pedas et al., 2008). Moreover, OsZIP1 has long been considered a metal uptake transporter for Zn and Cd in rice (Bashir et al., 2012; Ramegowda et al., 2013). Recent studies showed that over-expression of *OsZIP1* reduced concentrations of Zn, Cu, and Cd in rice and improved growth under metal stress (Liu XS et al., 2019). The function of *OsZIP1* is similar to that of the copper efflux transporter, *OsHMA9*, in which it has a Cd efflux function, causing an excretion of Cd from root cells and a reduction of Cd accumulation in rice (Lee et al., 2007). *OsZIP7* encodes a plasma membrane-localized protein with influx transport activity for both Zn and Cd, and plays an integral role in xylem loading in roots and inter-vascular transfer in nodes to preferentially deliver Zn and Cd to developing tissues and grains. Thus, knockout of *OsZIP7* resulted in the retention of Zn and Cd in roots and basal nodes (Ricachenevsky et al., 2018; Tan et al., 2019). Suppression of the zinc transporter genes *HvZIP3* and *HvZIP8* by RNA interference (RNAi) silencing showed increased Cd accumulation and reduced Zn and Mn concentrations in barley grains (Sun et al., 2015). However, considering that the metal concentrations used in some studies were much higher than those found in natural soils or rhizosphere environments, these conclusions should be viewed with caution. For example, the *RIT1* gene cloned from pea (*Pisum sativum* L.), a homolog of the *Arabidopsis IRT1* Fe transporter gene, was previously thought to be associated with Fe uptake, and induced a high rate of Cd^{2+} and Zn^{2+} influx into the roots of pea seedlings (Cohen et al., 1998). However, further research showed that the enhancing effect of *RIT1* on Zn and Cd existed only when these elements were present at elevated concentrations, but not at their physiologically relevant soil levels (Cohen et al., 2004).

P_{1B}-ATPases, also called heavy metal ATPases (HMAs), play direct roles in plant heavy metal transmembrane transport. *OsHMA2*, a transporter for Zn and Cd, is localized in the plasma membrane and mediates Zn/Cd xylem loading and intervascular transport to grains. However, studies showed that both *OsHMA2* knockout and overexpression plants had considerably reduced concentrations of Zn and Cd in leaves and grains compared with wild-type rice (Satoh-Nagasawa et al., 2012; Takahashi et al., 2012; Yamaji et al., 2013). *HMA2* genes have also been identified in other crops and have a highly conserved function among cereals. For example, barley *HvHMA2* has been identified predominantly in the plasma membrane and has a role in pumping Zn and Cd in yeast (Mills et al., 2012). Ectopic expression of *HvHMA2* in tobacco resulted in modified Zn, Fe, and Cd homeostasis (Barabasz et al., 2013). *TaHMA2* from bread wheat can transport Zn^{2+} and Cd^{2+} across membranes, and overexpression of *TaHMA2* in *Arabidopsis* increased Zn/Cd root-to-shoot translocation, and enhanced root length and fresh weight (Tan et al., 2013). In contrast, *OsHMA3* located in the tonoplast, plays a role in pumping Cd into vacuoles and has a decisive role in reducing the root-to-shoot translocation of Cd (Ueno et al., 2010; Miyadate et al., 2011). *HvHMA3* has been identified as a candidate gene for Cd accumulation in barley shoots and grains (Wu et al., 2015). In durum wheat, the *HMA3-B1* gene has been found to match perfectly with a major locus

Cdu1, which can explain 80% of the variation in grain Cd accumulation (Knox et al., 2009; Zimmerl et al., 2014). Sequence analysis of this *HMA3* gene in high- and low-Cd durum wheat cultivars showed that a premature stop caused by a 17-bp duplication occurred only in high-Cd cultivars. The close relationship between a severely truncated HMA3 transporter and high Cd phenotype further confirmed that *HMA3* was the best candidate gene for the *Cdu1* locus (Wiebe, 2012; Aprile et al., 2018).

OsHMA3 has high specificity for Cd rather than broad substrate-specificity. Consequently, loss-of-function or weak alleles of this gene result in increasing Cd root-to-shoot translocation (Ueno et al., 2010; Yan et al., 2016). In field trials, overexpression of *OsHMA3* decreased Cd concentrations in brown rice by more than 90%, but had no significant effect on grain yield or concentrations of other essential micronutrients, including Zn, Fe, Cu, and Mn (Sasaki et al., 2014; Lu et al., 2019). Given the fact that *OsHMA3* and *OsHMA2* have different tissue localizations and promoter activities, driving the expression of *OsHMA3* by *OsHMA2* promoter could specifically reduce Cd accumulation in grains, and mitigate the adverse effect on rice yield induced by *OsHMA2* knockout as well (Shao et al., 2018). These mutants might be used directly in breeding programs.

Recently, nodes in graminaceous plants such as rice and barley were found to play crucial roles in the allocation of multiple mineral nutrients, including essential and nonessential metal elements, by mediating intervascular transfers (Yamaji and Ma, 2014). Several node-expressed transporters, mainly in rice, have been found to transport Cd, and among them *OsHMA2* is expressed in the phloem of nodes (Satoh-Nagasawa et al., 2012; Takahashi et al., 2012; Yamaji et al., 2013). In addition, the plasma membrane-localized gene, low-affinity cation transporter 1 of rice (*OsLCT1*), is highly expressed in the uppermost node, and has been shown to mediate the export of phloem Cd and certain other elements into the sieve tube and translocate them from leaf blades and nodes to the grains of rice. Thus, down-regulation of *OsLCT1* resulted in an about 50% reduction of Cd in grains compared with that of control plants, whereas the contents of other metals and plant growth were little affected (Uraguchi et al., 2011, 2014). In addition, *OsCCX2*, a putative cation/Ca^{2+} exchanger (CCX), may function in loading Cd into xylem vessels, thus mediating direct root-derived Cd transport to grains. Knockout of *OsCCX2* caused a significant reduction of Cd content in rice grains (Hao et al., 2018). Shimo et al. (2011) found a novel gene, *OsLCD* (low Cd), which positively regulates Cd tolerance and accumulation in rice. A knockout via a T-DNA insertion reduced Cd accumulation by 43%–55% in grains, but had no effect on Cd accumulation in leaf blades. Yuan et al. (2012) reported that down-regulation of metal tolerance protein 1 of rice (*OsMTP1*, a bivalent cation transporter of heavy metals including Zn and Cd) markedly reduced heavy metal tolerance and accumulation in various rice tissues.

2.2 Chelation-related genes

In addition to transporter proteins, metal ligands and chelators including metallothioneins (MTs), phytochelatins (PCs), organic acids, and other cysteine (Cys)-rich peptides have a role in metal transport and homeostasis (Ovečka and Takáč, 2014). MTs belong to a superfamily of intracellular metal-binding proteins, and bind metals through their Cys residues. The isoform, *OsMTI-1b*, a rice MT type 1 gene, when expressed in yeast cells, results in increased tolerance to $CdCl_2$ treatment and increases accumulation of Cd^{2+} ions (Ansarypour and Shahpiri, 2017). PCs are predominantly associated with detoxification of non-essential toxic metals and metalloids such as Cd and arsenic (As), and thus significantly affect Cd accumulation in cereal plants (Das et al., 2017). Uraguchi et al. (2017) identified two independent *OsPCS1* mutant rice lines (a T-DNA and a Tos17 insertion line) and found that they exhibited increased sensitivity to Cd, showing the importance of *OsPCS1*-dependent PC synthesis for Cd tolerance in rice. Elemental analyses of rice plants showed decreased Cd accumulation in the grains of both mutants. Recently, a defensin-like protein, CAL1, was found to be involved in Cd accumulation in rice leaves. It promotes Cd secretion into the extracellular space via chelation, then loading into the xylem and long-distance transport, thereby resulting in most Cd being deposited in leaves rather than in grains. This provides a novel and ideal mechanism for developing dual-function rice cultivars that can produce low-Cd grains and be used as Cd hyper-accumulators for remediating contaminated soils (Luo et al., 2018).

3 Identification of molecular markers for low Cd accumulation in cereals

QTL mapping is acknowledged as an efficient approach for dissecting complicated agronomic traits controlled by multiple genes, and has been successfully applied to detect loci controlling Cd accumulation in some crops. A series of QTLs that control Cd concentrations have been reported in various plants including wheat, maize, barley, and rice (Table 2). Cd uptake, translocation, and accumulation in grains are complex processes that are controlled by many genes and are affected by environmental factors. Only a small number of genes related to low Cd accumulation have so far been identified, and the underlying knowledge regarding the molecular mechanisms for plant Cd uptake, translocation, and accumulation is still fragmental. Furthermore, the application of these genes to low-Cd plant breeding is still lacking and has little effect on cultivars released by breeders. The challenge now facing breeders is to identify more novel, effective low-Cd genes, and to successfully apply those already identified to produce low-Cd crop cultivars.

3.1 QTLs associated with Cd accumulation in rice

Ishikawa et al. (2005) detected three putative QTLs controlling Cd concentrations in brown rice located on chromosomes 3, 6, and 8. Kashiwagi et al. (2009) found that the interaction of two putative QTLs on chromosome 4 increased Cd concentrations in brown rice. Xue et al. (2009) identified six QTLs associated with Cd tolerance and three QTLs for root and shoot Cd concentrations in rice seedlings. Ueno et al. (2009b) identified a major QTL on chromosome 11 controlling the translocation of Cd from roots to shoots in seedlings that explained 16.1% of the detected variation in Cd accumulation. Ueno et al. (2009a) isolated another novel major QTL with a large effect on Cd distribution in roots and shoots of rice using an F_2 population derived from Anjana Dhan×Nipponbare. This QTL, detected on the short arm of chromosome 7, explained as much as 85.6% of the phenotypic variation. High-resolution mapping and complementation analysis identified *OsHMA3* as being the causal gene for this QTL locus (Ueno et al., 2010). Ishikawa et al. (2010) localized a major-effect QTL (named *qGCd7*) to the short arm of chromosome 7, which explained 35.5% of the observed variation, and enhanced grain Cd accumulation in rice grown in a Cd-polluted paddy field. Abe et al. (2011) detected a major QTL, *qCdp7* (accounting for 31%–54% of the phenotypic variance), on chromosome 7, that significantly increased Cd accumulation in both the grain and straw of rice. Zhang et al. (2011) identified three QTLs for Cd accumulation in brown rice. Sato et al. (2011) reported two QTLs for increasing Cd accumulation in brown rice: *qLCdG11* explained 9.4%–12.9% of the phenotypic variation, while *qLCdG3* accounted for 8.3%–13.9%. Yan et al. (2013) constructed a recombinant inbred (F_7) line (RIL) population to identify QTLs controlling Cd accumulation and distribution. Five significant QTLs were detected: *scc10* was responsible for Cd accumulation in shoots; *gcc3*, *gcc9*, and *gcc11* were closely associated with Cd accumulation in grains; and *sgr5* controlled the translocation of Cd from roots to shoots. Among them, *sgr5* had the greatest effect on the distribution of Cd in grains. Using 46 chromosome segment substitution lines (CSSLs), Abe et al. (2013) detected a major QTL, *qlGCd3*, on the long arm of chromosome 3, which was responsible for reduced Cd concentration in rice grains. Using 204 RILs, Hu et al. (2018) identified 22 QTLs affecting the accumulation of Cd, Cu, Fe, Mn, and Zn in brown rice, among which *qCd5* was a novel QTL that regulated low Cd accumulation. Luo et al. (2018) identified a QTL named *CAL1* (Cd accumulation in leaf 1) on chromosome 2 using a doubled haploid (DH) population derived from TN1 and CJ06. Cd levels in grains and leaves of the over-accumulating cultivar (TN1) were 3–4-fold higher than those in the under-accumulating cultivar (CJ06). The authors localized *CAL1* and cloned the causal gene using map-based cloning. Liu WQ et al. (2019) identified two QTLs (*qCd-2* and *qCd-7*) for Cd accumulation in rice grains. McCouch et al. (2016) established an open-access resource for genome-wide association studies (GWAS) in rice. Using this resource, Zhao JL et al. (2018) identified 14 QTLs for low Cd accumulation in rice grains evenly distributed in *indica* and *japonica* subpopulations consisting of 312 diverse rice accessions. These QTLs co-localized with functional genes, including *OsHMA3*, *OsNRAMP1*, *OsNRAMP5*, and *OsLCD*. Also, a novel QTL, *qCd3-2*, may encode a member of the rice NRAMP gene family (*OsNRAMP2*). Liu WQ et al.

Table 2 List of reported quantitative trait loci (QTLs) attributing to Cd accumulation in cereal crops

Crop	Mapping population	Maker	Traits	Chromosome	QTL	PVE (%)	Reference
Rice	39 CSSLs, Kasalath/ Koshihikari	129 RFLPs	Cd accumulation in grains	3, 6, 8	Putative QTLs for grain Cd		Ishikawa et al., 2005
Rice	98 BILs, Kasalath/ Nipponbare	RFLP and SSR	Cd concentration in leaves and culms	4, 11	*qcd4-1*, *qcd4-2*, *qcd11*		Kashiwagi et al., 2009
Rice	127 DHs, JX17/ZYQ8	RFLP and SSR	Cd concentration in roots and shoots or their ratio	1, 3, 5, 6, 7, 8, 10	4 QTLs		Xue et al., 2009
Rice	184 F_2, Badari Dhan/ Shwe War	141 SSRs	Cd concentration in shoots	2, 5, 11	A major QTL	16.1	Ueno et al., 2009b
Rice	177 F_2, Nipponbare/ Anjana Dhan	SSR	Root-to-shoot Cd translocation	7	A major QTL	85.6	Ueno et al., 2009a
Rice	965 F_2, Nipponbare/ Anjana Dhan	SSR	Cd concentration in shoots	7	*OsHMA3*		Ueno et al., 2010
Rice	85 BILs, Sasanishiki/ Habataki	SSR	Cd concentration in shoots and grains	2, 7, 12	*qGCd2*, *qGCd7*, *qSCd12*	7.2–35.5	Ishikawa et al., 2010
Rice	144 F_2, Cho-Ko-Koku 9 Akita 63	SSR	Root-to-shoot Cd translocation	7	*qCdT7*	88.0	Tezuka et al., 2010
Rice	F_2, Cho-Ko-Koku 9 Akita 63	CAPS	Root-to-shoot Cd translocation	7	*qCdT7*		Miyadate et al., 2011
Rice	103 BILs, Koshihikari/ Jarjan	169 SSRs	Cd concentration in grains	7	*qCdp7*	31.0–54.0	Abe et al., 2011
Rice	127 DHs, JX17/ZYQ8	RFLP and SSR	Cd concentration in grains	3, 4, 6	*qCdc3*, *qCdc4,* *qCdc6*	10.8–41.7	Zhang et al., 2011
Rice	126 RILs, Fukuhibiki/ LAC23	SSR and CAPS	Cd concentration in grains	3, 11	*gLCdG3*, *gLCdG11*	8.3–13.9	Sato et al., 2011
Rice	91 RILs, SNU-SG1/ Suwon490	124 SSRs	Cd concentration in shoots and grains	3, 5, 9, 10, 11	*gcc3, srg5, gcc9*, *gcc11*, *scc10*	16.1–24.9	Yan et al., 2013
Rice	46 CSSLs, Koshihikari/ LAC23	345 SNPs	Cd concentration in shoots	3	*glGCd3*		Abe et al., 2013
Rice	204 RILs, ZS97B/MY46		Cd concentration in grains	5	*qCd5*		Huang et al., 2018
Rice	DH, YK17/D50	170 SSRs	Cd concentration in brown or milled rice	2, 3, 4, 5, 7, 9	32 QTLs		Hu et al., 2018
Rice	119 DHs, 3651 BC3F3, Tainan1/Chunjiang06	RFLP	Cd concentration in leaves	2	*CAL1*	13.1	Luo et al., 2018
Rice	115 RILs, Xiang 743/ Katy	SSR	Cd concentration in grains	2, 7	*qCd-2*, *qCd-7*		Liu WQ et al., 2019
Wheat	155 DHs, W9262-260D3/ Kofa	SSR	Cd concentration in grains	5B	*Cdu1*		Knox et al., 2009
Wheat	DH, W9262-260D3/ Kofa	STS	Cd concentration in grains	5B	*Cdu1* and a minor QTL	80.0	Wiebe et al., 2010
Wheat	103 RILs, Ch/Sh		Cd concentration in roots	4A, 5D	2 QTLs	10.0–17.5	Ci et al., 2012

List of reported genes in rice (*Oryza sativa*) related to Cd homeostasis was reviewed by Chen JG et al. (2019). PVE: phenotypic variation explained; CSSL: chromosome segment substitution line; RFLP: restriction fragment length polymorphism; SSR: simple sequence repeat; BIL: backcross inbred line; RIL: recombinant inbred line; DH: doubled haploid; CAPS: cleaved amplified polymorphic sequence; SNP: single nucleotide polymorphism; STS: sequence tagged site

(2019) used 276 accessions with 416000 single nucleotide polymorphisms (SNPs) and performed GWAS of grain Cd concentrations in rice grown in heavily multi-contaminated farmland. Seventeen QTLs were found to be responsible for the grain Cd concentration. Yan et al. (2019) performed GWAS with 127 rice cultivars and identified a QTL on chromosome 3 that explained about 20.7% of the phenotypic variation. Using gene ontology (GO) and yeast spot analyses, they found a member of the major facilitator superfamily, *OsCd1*, that takes part in rice Cd uptake and grain Cd accumulation and was the causal gene for QTL3. These results provide a good reference for cloning potentially useful candidate genes and molecular breeding of low-Cd cereal crops.

3.2 QTLs associated with Cd accumulation in wheat

In contrast to multiple-gene control, durum wheat grain Cd accumulation seems to be controlled by a single dominant gene, *Cdu1*, which was first found on a linkage group using the random amplified polymorphic DNA marker (OPC20) (Penner et al., 1995). Later studies reported that its expression was highly heritable (0.84–0.88) and exhibited no significant effect on agronomic performance or the uptake of other micronutrients (Clarke et al., 1997, 2002). *Cdu1* is located on the long arm of chromosome 5B (Knox et al., 2009). The same major QTL, which is in the vicinity of *Cdu1*, has been repeatedly identified with different mapping populations and markers, explaining most of the Cd variation in durum wheat grain (Wiebe et al., 2010; AbuHammad et al., 2016; Salsman et al., 2018). With regard to the QTLs associated with Cd accumulation in vegetative organs in wheat, Ci et al. (2012) identified two QTLs for root Cd accumulation at the germination and seedling stages using 103 RILs derived from a cross of Ch×Sh. AbuHammad et al. (2016) also identified a major QTL contributing a low-Cd uptake allele and explaining 54.3% of phenotypic variation. Oladzad-Abbasabadi et al. (2018) identified a novel low-Cd uptake locus on chromosome 5BL, and the candidate causal gene was homologous to an aluminum-induced protein-encoded gene rather than to the closely linked *Cdu1-B* gene.

3.3 QTLs associated with Cd accumulation in maize and barley

In maize, Soric et al. (2009) detected a QTL associated with Cd uptake on chromosome 2, which could explain 49.8% of the phenotypic variation. Zhao XW et al. (2018) found 63 loci associated with leaf Cd accumulation through GWAS, and successfully identified a major QTL on chromosome 2 that contained 40 SNPs highly associated with leaf Cd concentration, which could explain over 38% of the phenotypic variation. In barley, Wu et al. (2015) detected nine QTLs for root Cd accumulation, 21 for shoot Cd accumulation, and 15 for grain Cd accumulation, via GWAS mapping of 100 accessions from the International Barley Core Selected Collection (BCS). Wang et al. (2019) identified a single QTL, *qShCd7H*, localized on chromosome 7H, responsible for shoot Cd accumulation, using a DH population derived from a cross between Suyinmai 2 (Cd-sensitive) and Weisuobuzhi (Cd-tolerant). Furthermore, a novel gene, *HvPAA1*, related to shoot Cd concentration, was identified from *qShCd7H*. Functional identification showed that *HvPAA1* plays a role in the detoxification of Cd in barley. The results provide a molecular basis for understanding Cd accumulation in barley and will contribute to the development of molecular markers that can be used in breeding for low-Cd-accumulating cultivars.

4 Breeding cereal cultivars with low Cd accumulation in grains

Although the use of agronomic practices and chemical regulators can reduce plant Cd uptake, it has been generally accepted that the development of crop cultivars with low Cd accumulation in edible parts is the most efficient approach to deal with medium or slightly Cd polluted farmland. Low-Cd-accumulating cultivars should accumulate Cd in edible parts only to a level below maximum permissible concentrations (MPC) for safe consumption, even when grown in Cd-contaminated soil. The MPCs are set by either the relevant national authorities or regional and international organizations. For example, the Cd standard limit for polished rice set by the Chinese National Standard (NHFPC, 2017) is 0.2 mg/kg DW, and the limit set by the Codex Alimentarius Commission (CAC, 2019) is 0.4 mg/kg DW. In addition to MPCs, further criteria have also been proposed for the selection of low-Cd cultivars. For instance, the yield and eating quality of selected low-Cd cultivars should not be reduced significantly when grown in Cd-polluted soils.

4.1 Methodologies for breeding for low-Cd cultivars

Breeding for low grain Cd cultivars can be achieved by both conventional and modern breeding approaches. Considerable genetic variation in Cd-accumulating ability has been widely reported in a range of cereal crops (Grant et al., 2008; Zhao JL et al., 2018; Zhao XW et al., 2018). Thus, in conventional breeding, low-Cd cultivars can be selected based on the determination of Cd content, coupled with measurements of morphological and physio-chemical parameters. To handle segregating accessions, such breeding strategies as mass, pure line, and recurrent selection methods can be effectively adopted in developing low-Cd cultivars. In addition, breeders adopt random cross combinations of genotypes to create genetic variation for screening low Cd accumulation genotypes. However, a more efficient strategy is to screen for low-Cd cultivars by measuring the grain Cd content of cultivars that have been or will be in commercial application (Chen et al., 2007a, 2007b; Cao et al., 2014b). Cultivars selected as low-Cd types from among existing commercial cultivars with excellent yield and agronomic traits can be directly used in commercial production. Conventional breeding methods have been partially successful in developing low-Cd cultivars. For example, a Cd-pollution-safe spring durum wheat cultivar named "Strongfield" was commercially released in Canada in 2004 (Clarke et al., 2006). However, this breeding method typically requires a large amount of effort, and a time-consuming selection process.

Recently, molecular breeding approaches have accelerated progress in breeding low-Cd cultivars by exploiting technologies such as marker-assisted selection, allele discovery, allele pyramiding, genome mutation, genome selection, genome-wide association mapping, and gene editing. The adoption of novel technologies, such as genetic modification, marker-assisted selection, and gene editing, has received increasing interest (Randhawa et al., 2013; Oladzad-Abbasabadi et al., 2018). Furthermore, the successful integration of molecular breeding with conventional breeding methods will accelerate the development of low-Cd cultivars.

4.2 Genetic differences and screening for low-Cd cereal cultivars—conventional breeding approaches

It is well documented that there are significant differences in Cd accumulation among genotypes. Hence, screening crop cultivars for low Cd content is a practical strategy for the selection of low-Cd cultivars and genotypes. This conventional breeding method has found its place in the development of low-Cd cereals. In rice, Arao and Ae (2003) investigated genotypic variation in grain Cd content, and identified three cultivars with the lowest Cd concentrations. Since then, many researchers have investigated the genotypic and environmental variation in Cd accumulation and distribution in rice and attempted to select low-Cd genotypes (Liu et al., 2005, 2007; He et al., 2006; Yu et al., 2006; Yan et al., 2010; Cao et al., 2014b; Pinson et al., 2015; Li et al., 2017; Zhou et al., 2017). In general, durum wheat tends to accumulate more Cd in grains than bread wheat (Stolt et al., 2003; Greger and Löfstedt, 2004). Wiebe et al. (2010) even suggested that the risk of high grain Cd threatening human health was limited to durum wheat. Thus, more attention and effort have been paid to breeding low-Cd durum wheat than bread wheat. Several dozen low-Cd durum wheat cultivars have been identified worldwide (Eriksson, 1990; Grant et al., 2008; Arduini et al., 2014; Zimmerl et al., 2014; Kubo et al., 2016; Perrier et al., 2016; Hirzel et al., 2017, 2018; Vergine et al., 2017; Oladzad-Abbasabadi et al., 2018; Yue et al., 2018).

Due to the similar chemical properties and electron structures of Zn and Cd (Järup and Åkesson, 2009; Nordberg, 2009), they often present in soils and move into plants via similar uptake and transport pathways (Waters and Sankaran, 2011; Khan et al., 2014). Thus, down-regulation of the pathways to reduce Cd accumulation in cereal grains may at the same time inadvertently decrease Zn transport (Palmgren et al., 2008; Sebastian and Prasad, 2014). Similarly, efforts to remove grain Cd during food processing by leaching and physical separation have reduced the content of some beneficial elements. For example, milling to produce white flour not only reduces Cd concentration by 50.0%, but also leads to reductions of 78.3% in Fe and 69.0% in Zn concentrations (Guttieri et al., 2015a). Fortunately, recent research on grain Cd and Zn contents in hard winter wheat germplasm indicated that their accumulation seems to be independently regulated by different regions of the genome, indicating that it may be possible to breed for low-Cd hard winter wheat genotypes without reducing Zn content (Guttieri et al., 2015b). In barley, our previous research successfully identified low grain-Cd barley genotypes such as Beitalys and Shang 98-128 (Chang et al., 1982; Wu and Zhang, 2002; Chen et al., 2007a, 2007b; Wu et al., 2007). There is also wide genetic variation in Cd content among maize cultivars. Hinesly et al. (1978) first reported large genetic variation in leaf and grain Cd concentrations among maize inbred lines, and found that hybrid maize line B73×R805 had the lowest Cd accumulation in grains when grown on sludge-amended soil (Hinesly et al., 1982). In a study of 19 inbred maize lines, a large genetic variation observed in shoot Cd concentration was attributed to root-to-shoot translocation rather than to Cd uptake (Florijn and van Beusichem, 1993a, 1993b). From evaluation of Cd concentrations (Zhang et al., 2008; Yang YM et al., 2014; Fahad et al., 2015; Retamal-Salgado et al., 2017), Chunyou30 was identified as a low-Cd maize genotype (Zhang et al., 2008). In short, conventional breeding methods have had partial success in the development of low-Cd cereals.

As they were selected from among commercial cultivars, these low-Cd genotypes can be used either directly or for hybridization to transfer the low Cd accumulation trait (Yu et al., 2006). However, with these breeding methods, breeders often prioritize the selection of low-Cd cultivars at the expense of yield and quality. Hence, some selected low-Cd cereal cultivars often exhibit reduced grain yield and lower nutritional quality (Chen et al., 2007a, 2007b). White and Broadley (2005) and Murgia et al. (2013) noticed a decrease in essential minerals such as Fe, Zn, Mn, and Ca in low-Cd cultivars. Fortunately, there are low-Cd cultivars with normal levels of essential minerals. Luo et al. (2018) evaluated 212 rice accessions, and identified CJ06 as a low-Cd rice cultivar. Further elemental analysis revealed that CJ06 contains levels of Fe, Mn, Zn, and Cu in grains similar to those in high-Cd-accumulating cultivars (i.e., TN1). However, it may be difficult to breed low-Cd cultivars when undesirable characters are linked to low-Cd-related genes, but problems of this type can now be overcome using novel technologies, including gene editing.

4.3 Molecular marker-assisted selection for low-Cd cereal cultivars

Molecular marker-assisted selection (MAS) should be a practical and acceptable alternative option. A typical example is the markers for a single major locus, *Cdu1*, related to Cd accumulation in durum wheat. The markers include a random amplified polymorphic DNA (RAPD) marker (OPC-20), an SNP marker which was able to identify 89% of the low-Cd lines in the Strongfield×Alkabo population, and two user-friendly competitive allele-specific polymerase chain reaction (KASP) markers (Cad-5B and Ex_c1343_2570756) for grain Cd accumulation, with an average prediction accuracy of 84%–88%. These markers have assisted the successful selection of low-Cd durum wheat (Knox et al., 2009; Wiebe et al., 2010; AbuHammad et al., 2016; Salsman et al., 2018). Likewise, *qShCd7H*, a major QTL controlling shoot Cd accumulation in barley, may facilitate the selection of low-Cd barley cultivars in the future (Wang et al., 2019). However, an insufficient number of validated markers have been the primary obstacle hindering the application of MAS to the selection of low-Cd progenies of staple food crops. Fortunately, the abundant germplasm resources of the main cereal crops has made GWAS possible, and with advanced linkage mapping technology, QTL identification and verification has become more powerful (Ueda et al., 2015; Nicod et al., 2016; Zhang et al., 2016). In addition, with the rapid development of next-generation sequencing and RNA sequencing (RNA-seq) profiling, the construction of high-density molecular markers and the quantification of expression profiles under Cd exposure have become possible (Wang et al., 2017; Aprile et al., 2018; Cao et al., 2019). Next generation sequencing is expected to enable the identification of further novel QTLs and molecular markers controlling Cd uptake, root-to-shoot translocation, and grain Cd accumulation.

4.4 Application of gene editing to breeding low-Cd cultivars

Progress has been made in the identification and cloning of low-Cd-related genes in the past two decades. For example, *AtIRT1* and *AtIRT2* (Connolly et al., 2002), *OsIRT1* and *OsIRT2* (Nakanishi et al., 2006), and *OsNRAMP1* (Curie et al., 2000) were identified and found to play roles in Cd uptake. Over-expression of *OsNRAMP1* in rice increased Cd accumulation in the leaves, indicating that *OsNRAMP1* was an important protein associated with high Cd accumulation in rice (Takahashi et al., 2011a). The knockout of *OsNRAMP5* or overexpressing *OsHMA3* significantly reduced Cd concentration in roots, shoots, and grains when compared with that of their wild-type relatives (Ueno et al., 2010; Sasaki et al., 2012, 2014; Yang M et al., 2014; Yan et al., 2016; Lu et al., 2019). The down-regulation of *OsLCT1* resulted in an about 50% reduction of Cd in grains compared with that of control plants (Uraguchi et al., 2011, 2014). Manipulating the expression of these transporter genes might be an efficient approach to reduce Cd content in grain crops. However, processes involved in the uptake and transport of Cd are closely associated with those involved in the uptake and transport of essential micronutrients such as Zn, Fe, and Mn (Nakanishi et al., 2006; Sasaki et al., 2012). Thus, there is still a great challenge, as reducing the Cd concentration in grains may simultaneously interfere with the balance of some essential mineral elements. One typical example is knockout mutations of the major transporter responsible for Mn and Cd uptake in rice—OsNramp5. These resulted in a large reduction of Cd in rice grains compared with that of wild-type plants (Ishikawa et al., 2012; Sasaki et al., 2012), but simultaneously, a decline in Mn concentration was detected (Ishikawa et al., 2012). Because it is an essential micronutrient for plant growth and development, Mn deficiency could inhibit growth and reduce yield. Similarly, the T-DNA insertion lines and RNAi lines of *OsNRAMP5* within a *japonica* cultivar Zhonghua 11, exhibited severe growth inhibition and a significant decrease in grain yield (grain yield of the T-DNA insertion mutant was only 11% of that of wild-type plants) (Sasaki et al., 2012). Supplying Mn fertilizer might alleviate Mn deficiency, but this problem may also be solved by inducing different mutation sites or using various genetic backgrounds. A good example is the *lcd-kmt* mutants of *OsNRAMP5* (produced by carbon ion-beam irradiation of a popular Japanese temperate *japonica* rice cv. Koshihikari), which show few adverse effects on growth with less than 100 mg/kg Mn in their straw (Ishikawa et al., 2012). Another example is a Zn and Cd transporter OsHMA2, which plays a role in Zn and Cd loading into the xylem and participates in root-to-shoot translocation of these metals in rice. Overexpression of *OsHMA2* driven by a *35S* promoter can significantly reduce both Zn and Cd contents in rice grains. However, unexpectedly, when *OsHMA2* was expressed under the control of *OsSUT1*, Cd concentration in the grains of SUT1-rice was only about half of that in the wild type, while the concentrations of Zn and other elements remained almost the same (Takahashi et al., 2012). The *OsSUT1* promoter is thought to enhance expression of the Zn transporter, improving its efficiency in accumulating Zn in rice grains (Scofield et al., 2007). In addition, *OsZIP7* seems to cooperate with *OsHMA2* to load Cd and Zn into sieve tubes in the phloem region of nodes, and thus OsSUT1-OsHMA2 is thought to be beneficial for reducing the Cd content of rice grains (Tan et al., 2019). However, further research is needed to determine whether OsSUT1-OsZIP7 constructs can reduce Cd concentrations in rice grains.

With advances in genomics and gene editing technology, molecular breeding for low Cd accumulation with high yield and quality may become a reality (Shan et al., 2014; Li et al., 2018). Tang et al. (2017) and Liu SM et al. (2019) demonstrated that it is feasible to generate yield-competitive and low-Cd rice lines through CRISPR/Cas9-mediated mutagenesis of *OsNramp5*, using the rice cultivars Huazhan and Xidao 1 as the parental wild-type lines. However, genetic modification does not always meet with favorable public perceptions (Chen JG et al., 2019; Chen KL et al., 2019).

Conventional and molecular breeding strategies, used alone and in combination, have been designed to develop low-Cd cereal cultivars to minimize Cd uptake and toxicity. Conventional breeding is still an attractive and potential tool for screening low-Cd crop cultivars. Molecular markers are a powerful technology for the selection of low-Cd cereal cultivars. In addition, modern molecular breeding technologies have great potential in breeding programs for the development of low-Cd cultivars, especially when coupled with conventional breeding. Within the next decade, the emphasis on low-Cd cereal breeding is likely to create more genetic variation for effective selection of low-Cd cultivars and further identification of unknown Cd accumulation-related genes and novel major QTLs controlling grain Cd accumulation. This will lead to a balanced use of phenotypic and genotypic selection and conventional and molecular breeding procedures. Both enhanced evaluation systems and regulations are necessary to determine whether low-Cd cultivars can be introduced into commercial production.

Contributors

Qin CHEN shaped the tables, wrote and edited the manuscript. Fei-bo WU wrote and edited the manuscript. Both authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

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Compliance with ethics guidelines

Qin CHEN and Fei-bo WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by either of the authors.

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中文概要

题　目：禾谷类作物低镉积累育种研究进展

概　要：镉（Cd）是毒性最强和农田受污染最普遍的重金属之一。土壤镉污染严重影响作物的产量和品质，并可通过食物链富集，从而危害人体健康。选育和种植食用部位镉低积累的作物品种是经济有效利用镉污染土壤，保证农产品安全生产，有效降低镉进入食物链的一条经济、有效的途径。本文主要介绍了近年来国内外在禾谷类作物镉低积累相关数量性状位点（QTL）定位、基因挖掘及其在低镉积累育种中的应用的最新成果；概述了镉低积累作物品种的定义、特征与选育方法，为低镉积累分子育种与生产提供理论指导。

关键词：禾谷类；低镉；数量性状位点（QTL）定位；育种