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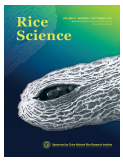

Review

Advances in Understanding Cadmium Stress and Breeding of Cadmium-Tolerant Crops

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Abstract: Cadmium (Cd) pollution has emerged as a critical global environmental concern, due to its significant toxicity, environmental persistence, and the pervasiveness of contamination. Significantly, the bioaccumulation of Cd in agricultural crops constitutes a primary vector for its entry into the human diet. This issue warrants urgent attention from both the scientific community and policymakers to develop and implement effective mitigation strategies. This review delves into the physiological impacts of Cd stress on plants, including the suppression of photosynthetic activity, amplification of oxidative stress, and disruptions in mineral nutrient homeostasis. Additionally, the resistance mechanisms deployed by plants in response to Cd stress have been explored, and the prospective contributions of molecular breeding strategies in augmenting crop tolerance to Cd and minimizing its bioaccumulation have been assessed. By integrating and analyzing these findings, we seek to inform future research trajectories and proffer strategic approaches to enhance agricultural sustainability, safeguard human health, and protect environmental integrity.

Key words: cadmium stress; crop tolerance; physiological response; molecular breeding strategy

Cadmium (Cd) pollution, due to its considerable toxicity, environmental persistence, and extensive contamination, represents a profound environmental challenge on a global scale (Jia et al, 2022). Human activities such as mining operations, power generation, industrial metallurgy, urban transportation, fertilizer application, and wastewater irrigation have precipitated a progressive buildup of Cd in terrestrial and aquatic ecosystems (Sarwar et al, 2010). The heightened solubility of Cd in soil matrices engenders deleterious impacts on crop yield and quality when cultivated in contaminated fields. Moreover, the biomagnification of Cd through the food chain, culminating in its absorption by crops and subsequent intake by humans, poses a grave risk to public health, necessitating immediate and effective restorative measures (Järup, 2003; Cao Z Z et al, 2018).

The fraction of Cd in the soil that is accessible for uptake by plants is referred to as bioavailable Cd. The assimilation of Cd from the soil into plants is modulated by a multitude of factors, among which soil pH is recognized as the most pivotal determinant. Acidic soil conditions typically enhance the solubility and absorbability, and thus the bioavailability of Cd for plant uptake. Empirical studies have delineated a linear correlation within a defined range, where a decrease in soil pH is invariably associated with a concomitant increase in the absorption of Cd by plants (Kim et al, 2009; Liu et al, 2015). However, it should be noted that some studies have reported that increased soil pH does not uniformly correspond with a reduction in plant Cd absorption (Eriksson, 1989; Xie et al, 2009). This indicates that beyond soil pH, additional variables also play a significant role in influencing the bioavailability of Cd. These factors include the concentration of mineral elements and the

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presence of organic matter within the soil matrix. Elements such as calcium (Ca), iron (Fe), and zinc (Zn) present in the soil compete with Cd for the same transport carriers, subsequently attenuating plant absorption of Cd (Nelson, 1986; Shao et al, 2008; Haider et al, 2021). Moreover, phosphate ions in the soil can bind with Cd to form precipitates, reducing the Cd content in the soil solution. Insoluble phosphates can also reduce the bioavailability of Cd (Dheri et al, 2007; Matusik et al, 2008). In addition, phosphorus (P) participates in the biosynthesis of the phytochelatins (PC) precursor glutathione (GSH) (May et al, 1998), equipping plants with an enhanced capacity to mitigate Cd-induced stress. Nitrate fertilizers have been demonstrated to promote Cd absorption by plants more than other forms of nitrogen (N) fertilizers (Yang et al, 2020). Conversely, ammonium fertilizers may contribute to a reduction in soil pH, thereby enhancing the uptake of Cd (Yu et al, 2006). However, it has been observed that increased use of nitrogen fertilizers can indirectly reduce Cd accumulation in crops through a dilution effect (Landberg and Greger, 2003). Soil organic matter, known to chelate metal cations, serves as the primary adsorbent for heavy metals within the soil matrix. Furthermore, organic matter can indirectly diminish the bioavailability of Cd by increasing soil pH levels (Ge and Hendershot, 2005; Hamid et al, 2019).

Biological determinants, including specific plant species and the secretion of root exudates, are crucial in modulating the bioavailability of Cd. A diversity in the propensity to absorb and accumulate Cd is apparent not only across various plant species but also within distinct ecotypes of the same species, indicating species-specific and intra-species variability in response to Cd exposure. For instance, wheat has been documented to accumulate Cd to a significantly greater extent compared with other cereal plants (Puschenreiter et al, 2005). Moreover, under both terrestrial and hydroponic cultivation, distinct Cd concentrations have been measured in the aerial parts and grains of the *indica* rice cultivar (Anjana Dhan) compared with the *japonica* rice cultivar (Nipponbare), exemplifying how genetic variation influences metal uptake and distribution (Ueno et al, 2009). Root exudates, consisting of secondary metabolites synthesized during photosynthesis, encompass a diverse array of compounds, including organic acids, proteins, peptides, and amino acids (Zulfiqar et al, 2019). These exudates possess the capability to chelate Cd ions within the soil matrix, thereby substantially reducing the extent of Cd uptake by plants (Sarwar et al, 2010; Haider et al, 2021).

Understanding the mechanisms of how plants resist or tolerate Cd stress is of crucial importance. Decades of research have revealed an intricate array of defense mechanisms employed by plants, ranging from physiological alterations to the activation of specific gene pathways. These findings offer profound insights into the intricate interplay between plants and their ambient environment, and serve as the foundation for the development of innovative strategies to address Cd stress. Molecular breeding stands at the forefront of these strategies. By harnessing advancements in genetics and biotechnology, scientists are endeavoring to cultivate crop varieties that are inherently resistant to Cd stress, thereby promoting sustainable agricultural methods and bolstering food security in the face of increasing environmental challenges.

This review endeavors to deliver a comprehensive examination of the impact of Cd contamination on crop viability, elucidate the resistance mechanisms plants invoke in response to Cd stress, and assess the promise of molecular breeding techniques as a viable solution to this critical agricultural issue.

Effects of Cd toxicity

Cd toxicity exerts a profound influence on plant physiology, representing a multifaceted interplay of biological responses. Upon Cd assimilation, plants initiate detoxification mechanisms to ameliorate Cd-induced stress. This adaptive response involves the sequestration and compartmentalization of Cd, predominantly facilitated by phytochelatin-mediated pathways as well as by its association with peptides, amino acids, and proteins (di Toppi and Gabbrielli, 1999; Abbas et al, 2018; Zhang et al, 2023). Nevertheless, with escalating external Cd concentrations or persistent exposure, plants manifest a spectrum of toxic symptoms. These deleterious symptoms include disruptions in the uptake and distribution of essential nutrients, diminished antioxidative defense capabilities, disturbances in cellular structure and function, compromised photosynthesis, transpiration, and respiration processes, as well as curtailed growth and maturation (di Toppi and Gabbrielli, 1999; Clemens, 2006; Ehsan et al, 2014; Zhang et al, 2019; Zhu et al, 2024). Consequently, pronounced negative changes in physiological parameters and overall growth performance are observed in affected plants.

Effect of Cd on nutrient minerals

Owing to its analogous chemical attributes, the

competition of Cd with binding sites of essential metal transporters and enzymes diminishes the plant's uptake of vital nutrients, including Fe, Zn, magnesium (Mg), copper (Cu), and manganese (Mn) (Verbruggen et al, 2009). Fe, serving as a cofactor and regulator of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX), is particularly susceptible to this competitive interference. Once absorbed by plants, Cd directly competes with Fe, leading to the manifestation of Fe deficiency symptoms, notably leaf chlorosis (He et al, 2017). In barley (*Hordeum vulgare*), exposure to 1.0 μmol/L of Cd notably diminishes the levels of P, potassium (K), Ca, Mg, Cu, Fe, Mn, Zn, molybdenum, and boron in the root tissues. However, the concentrations of these elements in the shoots remained unchanged compared with the control, suggesting a differential distribution of nutrients in response to Cd exposure (Guo et al, 2007). Furthermore, the provision of Cd in a dose-dependent manner results in a decrease in N, P, K, Fe, Zn, and Cu contents in bread wheat (*Triticum aestivum*), and affects all measured element contents (N, P, K, Ca, Mg, Fe, Cu, Zn, and Mn) of durum wheat (*Triticum durum*). These findings point to genotype-specific variations in sensitivity to Cd stress across different wheat cultivars (Eker et al, 2013). Cd competes with Zn, Fe, and Mn for binding to specific plasma membrane transport proteins, notably ZNT1 and MTP1, leading to a consequential decline in the absorption of these essential micronutrients (Küpper and Kochian, 2010). The knockout of the rice Mn/Cd transporter OsNRAMP5 leads to a substantial reduction in Cd accumulation, implicating the competitive inhibition of Cd on the absorption of divalent mineral elements, such as Ca, Mg, Cu, and Mn (Yang et al, 2019). Beyond its direct competition effects, Cd can also exert an indirect influence on the absorption and metabolism of essential nutrients through the disruption of plant physiological activities. In *Silene cucubalus*, Cd exposure reduces N absorption and assimilation by decreasing the activity of nitrate transporters and nitrate reductase (Tran and Popova, 2013). Cd treatment reduces the enzyme activity of nitrate reductase in soybean (*Glycine max*) roots, which correlates with reduced ammonia assimilation and N fixation, ultimately leading to a reduction in the formation of root nodules (Balestrasse et al, 2003). Furthermore, Cd inhibits the expression of *Arabidopsis thaliana* root *NRT1.1*, resulting in decreased root nitrate absorption (Mao et al, 2014).

The influence of Cd on the uptake of nutritional elements by crops constitutes a matter of substantial concern due to its potential to induce imbalances in crop nutrition, subsequently affecting both the health and yield of the crops. To ensure the quality of agricultural produce and food safety, it is imperative to delve a comprehensive investigation into the precise mechanisms and scopes of Cd's influence.

Cd induced oxidative damage

Upon entry into plant cells, Cd induces the accumulation of reactive oxygen species (ROS). While ROS at a lower concentration serve as signaling molecules in plants, their excessive accumulation can trigger oxidative damage and initiate programmed cell death. Cd^{2+} is not directly involved in cellular redox reactions and thus cannot generate ROS directly. However, it interferes with the photosynthetic apparatus by inhibiting the photoactivation of Photosystem II (PSII), specifically by suppressing electron transfer processes (Farooq et al, 2016). This leads to structural damage to essential organelles, such as mitochondria and chloroplasts, indirectly causing ROS production as a consequence of disrupted photorespiration pathways (la Rocca et al, 2009). In potato mitochondria, Cd^{2+}-induced H_2O_2 production *in vivo* appears to originate principally from the mitochondrial electron transfer, as Cd^{2+} has been shown to elevate ROS production in isolated mitochondria (Heyno et al, 2008). Furthermore, Cd also prompts the activation of membrane-associated NADPH oxidase within peroxisomes, which results in ROS production (Tran and Popova, 2013). Under Cd stress, ROS generation occurs not only because of elevated electron flow within the mitochondrial and chloroplast electron-transport chain but also within peroxisomes, plasma membranes, nuclei, and apoplasts (Romero-Puertas et al, 2019). Additionally, Cd exposure adversely impacts the plant's ability to detoxify ROS: Cd^{2+} replaces Zn^{2+} in the enzyme Cu/Zn-SOD, consequently altering the enzyme conformation, rendering it dysfunctional, and leading to its degradation (Xu et al, 2009). Enhanced ROS production was noted in Scots pine (*Pinus sylvestris*) seedlings treated with 50 mmol/L Cd for a period of 6 h (Schützendübel et al, 2001). Similarly, in *Arabidopsis thaliana*, after 8 d of treatment with 10 μmol/L Cd, there was an approximate doubling of H_2O_2 content within the roots (Jin et al, 2013). Comparative analyses in mung beans, sunflowers, and peas have indicated that Cd toxicity reduces the

enzymatic activities of superoxide dismutase (SOD) and CAT, two key antioxidants responsible for mitigating oxidative stress within plants (Somashekaraiah et al, 1992; Fodor et al, 1995; Sandalio et al, 2001).

Nevertheless, in response to Cd stress, plants enhance the activity of antioxidant enzymes as a primary defensive mechanism to suppress ROS production. Antioxidant defense systems in plants can be classified into enzymatic and non-enzymatic antioxidants. The enzymatic group includes SOD, peroxidase (POD), CAT, APX, γ-glutamyl cysteine synthetase, glutathione synthetase, dehydroascorbate reductase (DHAR), glutathione reductase, among others. Non-enzymatic antioxidants encompass GSH, free proline, ascorbic acid (AsA), flavonoids, phenols, and additional compounds. In wheat seedlings and roots, the activities of SOD, CAT, and POD increase in correlation with the elevated soil Cd concentrations, reaching a maximum before declining as intraplant levels of Cd rise (Chen et al, 2014). The activity of glutathione S-transferase in wheat also intensifies under Cd stress (Wang et al, 2011). Furthermore, Cd exposure promotes the production of GSH and associates with an increase in the metabolites of the AsA-GSH cycle in wheat roots (Paradiso et al, 2008; Ahmad et al, 2009). Similarly, Cd treatment elevates the activity of DHAR in rapeseed and augments proline levels in tobacco, underscoring a conserved pattern of engaged antioxidant defense mechanisms across species in the presence of Cd stress (Islam et al, 2009; Markovska et al, 2009).

Excessive accumulation of ROS can instigate the peroxidation of proteins and lipids. Such lipid peroxidation within membrane components not only causes a decline in polyunsaturated fatty acids but also impairs the fluidity of membrane lipids, leading to membrane dysfunction. This dysfunction is characterized by cellular transparency lesions and fibrosis, culminating in cell lysis (la Rocca et al, 2009; Younis et al, 2016). Recent findings suggest that, after 24 h of Cd stress, there is a notable reduction in the concentrations of various lipid constituents in wheat roots, including digalactosyldiacylglycerol, monogalactosyldiacylglycerol, phosphatedylcholine, phosphatidylethanolamine, phosphatidylserine phosphatidylglycerol, and lysophosphatidylcholine (Wu et al, 2022). Moreover, the excessive production of ROS can damage the molecular structure of DNA, with mitochondrial and chloroplastic DNA being particularly susceptible to ROS-induced damage due to their lack of protective histones and proximity to ROS production sites. Over-accumulation of ROS can also delay DNA replication and base repair processes (Mittler et al, 2004). In *Arabidopsis*, Cd stress inhibits the expression of three key components of DNA mismatch repair: MSH2, MSH6, and MLH1, highlighting the potential genotoxic effects of Cd (Cao X et al, 2018).

Cd stress in plants elicits a multifaceted physiological response, significantly involving the production of ROS and triggering a robust defensive response. The balance between ROS production and the plant's defense mechanisms is crucial in determining the overall impact of Cd stress. This delicate equilibrium highlights the intricate interplay between exogenous stress factors and the plant's internal defense systems. Disruptions in this balance may result in significant consequences for plant health, influencing growth parameters and overall yield.

Cd toxicity affects photosynthesis

Impaired photosynthetic activity is recognized as a defining symptom of Cd toxicity in plants. Exposure to Cd detrimentally affects both the light and dark reactions, as well as the photochemical efficiency of the photosystems, culminating in diminished photosynthetic efficacy. Furthermore, Cd hampers the synthesis of chlorophyll and disrupts enzymatic activities associated with its biosynthesis. ROS-induced damage compromises the structural and functional integrity of chloroplasts, precipitating the degradation of chlorophyll. This inhibitory effect of Cd on chlorophyll biosynthesis has been documented in maize leaf segments and can be mitigated by the application of reduced glutathione, illustrating the role of glutathione status in modulating Cd-induced phytotoxicity (Parekh et al, 1990). In wheat seedlings, Cd stress adversely impacts growth and photosynthetic capability, leading to a reduction in chlorophyll content and other photosynthetic pigments, such as chlorophyll a, chlorophyll b, and carotenoids, with these detrimental effects exhibiting a dose-dependent intensity (Ci et al, 2010). In sunflower (*Helianthus annuus*), increasing Cd concentration corresponds with a gradual decline in both chlorophyll content and the efficiency of PSII (as indicated by the chlorophyll fluorescence F_v to F_m ratio). Notably, the diminishment of chlorophyll b content is more marked than that of chlorophyll a (Azevedo et al, 2005). Cd also suppresses the activities of crucial enzymes associated with the Calvin cycle, notably ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenol-pyruvate carboxylase. It damages the structure of

RuBPCase directly by replacing the essential cofactor Mg^{2+} in the carboxylation reaction. Furthermore, Cd induces an irreversible dissociation of the large and small subunits of RuBPCase. This enzymatic dysfunction culminates in a significant impediment to the leaf's carbon fixation capabilities, particularly in the assimilation of CO_2 (Tran and Popova, 2013; Noor et al, 2018). Fe^{2+} plays an integral role in the biosynthesis of photosynthetic pigments and is intricately involved in the electron transfer processes within both PSI and PSII (Rochaix, 2011). It also functions as a pivotal cofactor for RuBPCase (Hasan et al, 2009; Saxena et al, 2016). Cd exerts its inhibitory effect by suppressing the activity of Fe^{3+} reductase, leading to a deficiency in Fe^{2+}, which in turn diminishes photosynthetic rate. Moreover, Cd stress induces stomatal closure in leaves, accompanied by reduced epidermal cell size and decreased stomatal density (Younis et al, 2016; Abbas et al, 2018; Rossini et al, 2022). Such alterations impinge upon the plant's ability to perform gaseous exchange efficiently and, in conjunction, further inhibit photosynthetic processes.

Inhibition of plant growth and development by Cd toxicity

Manifestations of Cd toxicity in plants are readily evident through notable changes in plant morphology, alongside inhibited growth and developmental processes. Roots, as the primary contact points for Cd uptake and also the tissue with the highest Cd content in plants, are particularly susceptible to its toxicity. Cd stress causes root tips to brown, restricts root elongation, and leads to abnormal morphological development. Prolonged Cd exposure leads to root necrosis and decay, eventually resulting in plant death (Ci et al, 2009; Abbas et al, 2018). A common hallmark of Cd toxicity is restriction, an effect largely attributable to Cd's inhibitory influence on cell elongation and division (White and Brown, 2010; Lux et al, 2011). Cd inhibits the mitosis of meristematic cells, leading to abnormal enlargement of cortical cells and epidermal apical regions, thereby reducing root length and lateral root number while simultaneously increasing root diameter (Krantev et al, 2008; Zhang D Z et al, 2022). Cd causes obstructions in root elongation and alterations in root morphology across a variety of crops, including rice, wheat, and peas (Rizwan et al, 2012, 2016; Tran and Popova, 2013). Cd damages the ultrastructure of chloroplasts, disrupts pigment molecular structures, and inhibits pigment biosynthesis, leading to the yellowing of leaves in crops such as rapeseed (Baryla et al, 2001; Miyadate et al, 2011). In wheat, excessive Cd correlates with reductions in leaf number and leaf surface area (Khan et al, 2007). Through its impact on root systems and aerial tissues like leaves, Cd ultimately results in a decrease in plant biomass. Reproductive functions are also adversely affected. Cd can interfere with pollen development, leading to aberrant pollen morphology, germination difficulties, and alterations in pollen tube growth (Xiong and Peng, 2001; Wang et al, 2014). Cd toxicity is also manifested in seed development, causing a noted decrease in the contents of lipids, proteins, and carbohydrates in soybean seeds, leading to a significant reduction in the quality of mature seeds (Malan and Farrant, 1998). In rice grains, Cd has been found to reduce the contents of carbohydrates and amino acids (Zeng et al, 2021). Cd toxicity is frequently associated with the inhibition of seed germination, representing a significant impediment to the initiation of plant life cycles. Under Cd stress, the activities of α-amylase, dehydrogenase, and nitrate reductase in seeds are reduced. This enzymatic suppression results in decreased soluble sugar levels, obstructed energy metabolism, reduced efficiency in nitrate utilization, and a decline in amino acid and protein contents, which further affects seed germination (Liu et al, 2007; Kalai et al, 2016). In the crop cowpea (*Vigna unguiculata*), Cd has been observed to inhibit both the absorption of water by seeds and the enzymatic activities responsible for starch hydrolysis. This dual inhibition restricts the transport of water and essential nutrients to the embryo, leading to a reduction in seed germination rate (Vijayaragavan et al, 2011). Additionally, Cd can interfere with germination by competing with Ca ions for binding sites on calmodulin, as evidenced in the germination of radish seeds (Huybrechts et al, 2019).

Mechanisms of Cd tolerance in crops

As a toxic heavy metal prevalent in soils and the environment, Cd exerts a detrimental influence on plant growth and development, thereby constraining both the yield and quality of crops (Dalcorso et al, 2010). Consequently, a comprehensive understanding of plant tolerance mechanisms in response to Cd is crucial for ensuring agricultural productivity and food security. To restrict the cellular ingress of Cd, plants regulate the expression of specific transporters. Heavy metals are meticulously regulated and partitioned to

curtail their concentrations in essential tissues and organelles, thus attenuating Cd toxicity (Uraguchi et al, 2009). In addition, plants generate hormones, proteins, signaling molecules, and antioxidants associated with stress, bolstering their capacity to cope with stress and maintain internal homeostasis, which in turn mitigates or even nullifies the adverse impacts of Cd (Noor et al, 2022).

Accumulation and distribution of Cd

The resilience of plants to Cd stress is intricately related to the mechanisms governing its uptake, accumulation, and systemic distribution within plant tissues. Upon assimilation by crop roots, Cd ions initiate their transport and movement within the plant. Predominantly, Cd is transported from the root system to aerial parts via the xylem, then redistributed in the leaves and stems, and enters the seeds through the phloem (Ai et al, 2022). Within plant cells, Cd is sequestered by chelators and compartmentalized in vacuoles as a means to diminish the cytosolic concentration of bioavailable Cd, thereby mitigating its phytotoxicity. The uptake and translocation of Cd are facilitated by pathways that also serve the absorption and movement of essential nutritional elements. To date, research in species such as rice, *Arabidopsis*, and yeast has identified five types of transport proteins involved in Cd dynamics: NRAMP (natural resistance-associated macrophage protein), HMA (heavy metal transporting ATPase), ABC (ATP-binding cassette) superfamily, ZIP (zinc and iron-regulated transporter protein), and YSL (yellow stripe-like protein).

Cd uptake in plants commences at the root system, facilitated predominantly by transport proteins located on the plasma membranes of root cells. These transporters are members of the NRAMP family, notably OsNRAMP1 and OsNRAMP5 in rice, which play critical roles as important Mn/Cd transport proteins. Targeted knockout of *OsNRAMP1* or *OsNRAMP5* in rice has demonstrated a significant reduction in Cd accumulation. Notably, the ablation of *OsNRAMP5* does not impact rice yield (Takahashi et al, 2011; Tang et al, 2017). In *Arabidopsis*, the knockout of the *AtNRAMP3* gene results in enhanced resistance to Cd, whereas plants overexpressing the *AtNRAMP3* gene are more sensitive to Cd stress (Thomine et al, 2000), suggesting that AtNRAMP3 facilitates the absorption of cadmium in plants (Thomine et al, 2003). In Polish wheat (*Triticum polonicum*), overexpression of *TpNRAMP5* significantly increases the Cd concentration in roots, shoots, and whole plants (Peng et al, 2018). Similarly, HvNRAMP5 in barley is located on the plasma membrane of root tip epidermal cells and is essential for Cd uptake, as evidenced by reduced tissue Cd content following *HvNRAMP5* knockout (Wu et al, 2016). In yeast expression systems, the iron transporters OsIRT1 and OsIRT2 from rice exhibit Cd influx transport activity (Ishimaru et al, 2006). Yeast expressing *OsIRT1* and *OsIRT2* became more sensitive to Cd, suggesting that Cd was absorbed by OsIRT1 and OsIRT2 (Nakanishi et al, 2006). IRT1 in *Arabidopsis thaliana* is also capable of mediating Cd absorption (Vert et al, 2002). Furthermore, *OsCd1*, identified through QTL mapping as a Cd transport-related gene, belongs to the major facilitator superfamily and is located on the cell membrane, implicated in the mediation of Cd uptake in rice roots and Cd accumulation in grains. Loss-function-of *OsCd1* or carrying the natural variant Val449Asp links to reduced Cd accumulation, elucidating varietal differences in Cd content between *indica* and *japonica* rice varieties (Yan et al, 2019). The Zn iron transport protein ZIP family, including OsZIP5 and OsZIP9, also participates in the process of root uptake of Cd. The rice *oszip5oszip9* double mutant has a significantly lower ability to absorb Cd compared with the wild type (Tan et al, 2020).

Part of the Cd ions that penetrate the root cell cytoplasm are rapidly engaged in chelation reactions with various organic molecules to mitigate their potential interference with cellular metabolic activities. These chelates include plant chelating peptides (PCs), metallothioneins, GSH, organic acids, and amino acids, among others. Concurrently, to alleviate the cytotoxic burden of heavy metals in the cytoplasm or other important cellular components, compartmentalizing Cd ions or Cd ion chelates into vacuoles is an important detoxification mechanism (Clemens, 2006).

Upon exposure to Cd, plants activate their defense mechanisms by synthesizing PCs, which then chelate with Cd^{2+} to form low molecular weight (LMW) complexes. These LMW complexes are transported into the vacuole through the transport protein HMT1 (an ATP-binding cassette-type transport protein) on the vacuolar membrane. Once sequestered within the vacuolar compartment, LMW forms high molecular weight complexes (HMW) with Cd or sulfides present. HMW is compartmentalized within the vacuole, reducing the concentration of free Cd^{2+} in the cytoplasm,

thereby alleviating the toxic effects of Cd on plants (Ortiz et al, 1995; Heiss et al, 2003).

In *Arabidopsis*, ABCC1, ABCC2, and ABCC3 are located on the vacuolar membrane of root cells, playing key roles in sequestering PC-Cd complexes into vacuoles. The *abcc1abcc2* double mutant and *abcc3* mutant show increased sensitivity to Cd stress, while overexpression of *ABCC3* in the *abcc1abcc2* mutant restores Cd tolerance, highlighting the importance and functional redundancy of these proteins in Cd distribution and accumulation (Park et al, 2012; Brunetti et al, 2015). Additionally, metallothioneins and GSH bind Cd to form chelates (Cobbett and Goldsbrough, 2002; Hassinen et al, 2011), which are sequestered in vacuoles to mitigate toxicity, though the specific transport proteins remain unidentified (Freeman et al, 2004; Zhang J L et al, 2022).

OsHMA3 plays a fundamental role in the dynamics of Cd trafficking from rice roots to vacuoles. It reduces the proportion of Cd transported to the aerial tissues, thereby enhancing the plant's tolerance to Cd (Ueno et al, 2010). In barley, the expression of *HvHMA3* is regulated by the promoter region containing a Sukkula-like transposable element, affecting the accumulation of Cd (Lei et al, 2020). In soybeans (*Glycine max*), the corresponding gene *GmHMA3* encodes a protein located in the endoplasmic reticulum of root cells, responsible for storing Cd in the endoplasmic reticulum, thus impeding its transport to shoots and ultimately diminishing the accumulation of Cd in seeds (Wang et al, 2012, 2018). Metal tolerance protein 1 (MTP1), as a member of the CDF (Cation Diffusion Facilitator) family, is involved in the compartmentalization, storage, and translocation of metal ions inside vacuoles (Krämer et al, 2007). Ectopic expression of *OsMTP1* enhances Cd resistance in yeast. Moreover, rice *OsMTP1* silenced using RNA interference is more sensitive to Cd, and the aerial parts have lower Cd content than the wild type. OsMTP1 is also present in sieve tube cells of leaves, suggesting a role in the regulation of Cd from the roots to shoots as well as within the phloem of leaves (Yuan et al, 2012).

Through processes of precipitation, chelation, and compartmentalization, the root system concentrates significant amounts of Cd^{2+}, subsequently diminishing the translocation of Cd^{2+} to aerial parts. With the continuous increase in Cd^{2+} stress concentration and duration, the roots become overloaded. Subsequently, Cd^{2+} is transported from the roots via the xylem to the aerial stems and leaves, undergoing redistribution at the stem nodes. Following that, it is translocated by the phloem to various plant tissues, including seeds, where it may accumulate (Takahashi et al, 2012). In rice, OsHMA2 represents a category of metal transport proteins expressed on the cell membrane that functions in efflux, and it primarily facilitates the uptake of Cd^{2+} and Zn^{2+} into the xylem. In the *Oshma2* mutant, the Cd content in the leaves and grains is significantly reduced, highlighting the transporter's significant role in Cd distribution within the plant, particularly to the seeds (Satoh-Nagasawa et al, 2012; Yamaji et al, 2013). Similar functions have been observed in wheat and barley for their respective homologs, TaHMA2 and HvHMA2, which are involved in the transport of Cd from the roots to the stems (Mills et al, 2012; Tan et al, 2013; Qiao et al, 2018).

Uraguchi et al (2011) cloned a transporter gene *OsLCT1* in rice, which encodes a transporter that is highly expressed in the vascular bundles of rice stem nodes. The expression of this gene is pronounced in the leaves and stem nodes during the reproductive stage, and it plays a pivotal role in the modulation of Cd transportation and accumulation in the aerial parts of rice, predominantly via the phloem. Additionally, during the grain maturity stage, OsLCT1 acts as the crucial transporter facilitating the movement of Cd from stem nodes to the seeds. The *Cadmium accumulation in leaf 1* (*CAL1*) gene, representing a rice QTL on chromosome 2, encodes a protein localized to the cell walls of both rice roots and leaf sheaths (Luo et al, 2018; Zhao and Huang, 2018). The CAL1 binds Cd^{2+} in the cytoplasm, forming complexes that are subsequently secreted into the apoplast, especially in the xylem parenchyma cells, thereby facilitating the efficient loading of CAL1-Cd complexes into the xylem vessels for translocation to the above-ground tissues (Zhao and Huang, 2018). Due to the binding of CAL1 with Cd^{2+}, the transport of Cd chelates to the phloem is organized, thus blocking the transport of Cd to the grains (Luo et al, 2018).

In rice, the Cation/Ca Exchangers 2 (OsCCX2) is a transporter that localizes to the plasma membrane and is primarily expressed in the xylem region of the nodal vascular tissues. Knockout of *OsCCX2* significantly reduces Cd content in the grains. Further studies have shown that the disruption of this gene leads to a decrease in the Cd translocation ratio from the roots to the shoots. It is postulated that OsCCX2 functions as an efflux transporter, playing a pivotal role in

facilitating the loading of Cd into xylem vessels (Hao et al, 2018).

The ZIP family members, OsZIP3, OsZIP6, and OsZIP7, have been implicated in the Cd transport process in rice, from the roots to the aerial parts and through the phloem mediation. OsZIP6 is hypothesized to localize on the plasma membrane in root and shoot tissues, and is involved in Cd transport from roots to shoots (Kavitha et al, 2015; Zhang J L et al, 2022). OsZIP7, in particular, plays an integral role in xylem loading in roots and inter-vascular transfer in nodes to preferentially deliver Zn and Cd to develop tissues and rice grains by cooperating with OsZIP3 and OsHMA2 (Sasaki et al, 2015; Tan et al, 2019; Ai et al, 2022). Furthermore, the co-expression of OsLCT1, OsHMA2, and OsZIP3 can effectively decrease the translocation and accumulation of Cd to rice grains (Tian et al, 2019).

Enhancing antioxidant capacity

Under conditions of Cd stress, the metabolism of ROS in plants is impeded. Hydroxyl (·OH), H_2O_2, superoxide anion ($O_2^{\cdot-}$), and other ROS accumulate in large amounts, leading to lipid peroxidation in the cell membrane and disruption of electron transport, thereby inflicting oxidative damage on the plants (Rahman et al, 2016; Considine and Foyer, 2021). To counteract or mitigate the oxidative insult induced by Cd, plants have evolved sophisticated antioxidant enzyme defense systems, which include (1) enzyme systems: CAT, POD, SOD, APX, GPX, GR, DHAR, peroxiredoxins, thioredoxins, and glutaredoxins, (Kohli et al, 2019); (2) non-enzymatic systems: antioxidants such as GSH, AsA, flavonoids, anthocyanin glycosides, etc. (Schützendübel and Polle, 2002; Dai et al, 2012). Through the synergistic augmentation of antioxidants and the activation of antioxidative enzymes, plants bolster their resistance to Cd-induced disturbance (Angulo-Bejarano et al, 2021). Melatonin, as a multifunctional molecule, has been shown to interface with both enzymatic and non-enzymatic systems to reestablish redox homeostasis and alleviate the oxidative stress induced by Cd exposure (Gu et al, 2021). Simultaneously, plant cells synthesize osmoregulatory substances such as amino acids, betaine, polyamines, mannitol, and trehalose to sustain regular cellular growth and to keep the stability of the cell membrane intact (Vinocur and Altman, 2005).

Promoting Cd efflux

The efflux of Cd from cellular interiors is a critical mechanism that directly diminishes its intracellular accumulation. Current studies suggest the absence of a specific transporter dedicated to Cd, and Cd efflux is typically achieved by transporters with primary affinities for other heavy metals. The P_{1B}-ATPase is a crucial class of metal expulsion transporter involved in the active extrusion of metal ions. OsHMA9, located on the plasma membrane, functions as a heavy metal efflux protein, which can alleviate the toxicity of high concentrations of Cd, Cu, and Zn in rice (Lee et al, 2007). Another transporter, OsABCG36, also located on the plasma membrane, plays a crucial role as a Cd efflux pump. Knockout studies of *OsABCG36* result in elevated Cd accumulation in the root cell sap and heightened Cd sensitivity compared with the wild type (Fu et al, 2019). OsZIP1, another type of metal efflux transport protein, predominantly associates with the root's endoplasmic reticulum and plasma membrane, is implicated in improved plant growth under excess metal stress and a concomitant decrease in metal uptake in transgenic rice overexpressing OsZIP1 (Liu X S et al, 2019). *Arabidopsis thaliana* exhibits other carriers that mediate the transfer of Zn or Cd, such as Plant Cadmium Resistance 1 (PCR1) and Plant Cadmium Resistance 2 (PCR2) (Song et al, 2004; Reddy et al, 2023), as well as G-type ABC Transporter 36/Pleiotropic Drug Resistance 8 (ABCG36/PDR8) (Kim et al, 2007; Sheng et al, 2019). These transporters collectively contribute to mitigating Cd toxicity by facilitating the extrusion of Cd from the cells, leading to a reduction of its cytosolic concentration (Table 1).

Regulation of gene expression

As delineated in prior discussion, many channel proteins are involved in the accumulation and distribution of Cd within plants. A salient strategy for modulating Cd transport activity in plants under heavy metal Cd exposure entails the transcriptional regulation of gene expression. The adaptive response to Cd stress is underscored by alterations in the expression profiles of numerous genes integral to the plant's resistance mechanisms. Transcription factors, as pivotal regulatory elements, exert profound influence over the gene networks that confer plant resilience to Cd stress. Research has identified several genes encoding transcription factors that are implicated in the orchestration of plant responses to Cd, thereby elucidating the genetic foundations of plant defense strategies against heavy metal toxicity. The transcription factor FIT (FER-like

Table 1. Key transporter genes involved in cadmium (Cd) stress response in various plant species.

Gene/Transporter	Function/Role	Reference
AtNRAMP3	Cd absorption in *Arabidopsis*	Thomine et al, 2000
PCR1, *PCR2*	Cd transfer in *Arabidopsis*	Song et al, 2004
OsIRT1, *OsIRT2*	Cd influx transport in rice	Ishimaru et al, 2006
ABCG36/PDR8	Cd efflux in *Arabidopsis*	Kim et al, 2007
OsHMA3	Cd trafficking from rice roots to vacuoles	Ueno et al, 2010
OsLCT1	Cd transportation and accumulation in rice	Uraguchi et al, 2011
OsNRAMP5	Mn/Cd transport in rice	Takahashi et al, 2011
OsHMA2	Cd and Zn transport to aerial parts in rice	Yamaji et al, 2013
OsZIP3	Cd transport from roots to shoots in rice	Sasaki et al, 2015
OsZIP6	Cd transport from roots to shoots in rice	Kavitha et al, 2015
OsZIP7	Cd transport to developing tissues and grains in rice	Sasaki et al, 2015
HvNRAMP5	Cd uptake in barley	Wu et al, 2016
OsNRAMP1	Mn/Cd transport in rice	Tang et al, 2017
CAL1	Cd binding and secretion in rice	Luo et al, 2018
OsCCX2	Cd loading into xylem vessels in rice	Hao et al, 2018
TpNRAMP5	Cd concentration increase in Polish wheat	Peng et al, 2018
OsABCG36	Cd efflux pump in rice	Fu et al, 2019
OsCd1	Cd uptake in rice root and accumulation in grain	Yan et al, 2019
OsZIP5, *OsZIP9*	Cd root uptake in rice	Tan et al, 2020

iron deficiency-induced transcription factor), alongside bHLH transcription factors bHLH38 and bHLH39, has been implicated in plant responses to Cd stress. Studies have shown that the transcription levels of the three aforementioned genes increase under Cd stress conditions, and the transgenic plants overexpressing either FIT/bHLH38 or FIT/bHLH39 exhibit enhanced Cd resistance (Wu et al, 2012). The rice PHD-finger protein OsTITANIA, acts as a constitutively expressed regulator of multiple metal transporter genes, responsible for essential metals delivery to shoots for normal growth (Tanaka et al, 2018). An additional transcription factor of note is WRKY13, which activates *PDR8* expression, thereby exerting a positive regulation of Cd tolerance in *Arabidopsis* (Sheng et al, 2019). Other critical transcription factors engaged in the Cd stress response include CaPF1, PvERF15, PvMTF-1, ZmOXS2, and HsfA4a (Fig. 1) (Tang et al, 2005; Shim et al, 2009; Sun et al, 2015; He et al, 2016; Lin et al, 2017).

Beyond transcription factors, microRNAs (miRNAs) also play pivotal roles in the gene regulatory network under Cd stress. miR390 has been shown to influence rice tolerance and Cd accumulation, with its expression being upregulated following Cd exposure. Overexpression of miR390 results in decreased Cd tolerance and increased Cd accumulation, along with increased expression levels of *OsNRAMP5* and *OsHMA2*, suggesting that miR390 acts as a negative modulator of Cd stress tolerance in rice (Ding et al, 2016). Furthermore, miR166, which targets *OsHB4*, has been associated with Cd sensitivity and accumulation in rice leaves and grains. Overexpression of *OsHB4* increases these effects, while miR166 counteracts Cd accumulation by degrading *OsHB4* transcripts (Ding et al, 2018).

Several novel genes have been isolated through the screening of rice mutants for Cd tolerance. OsLCD (low-Cd), a novel protein with no homology to other known proteins and localized to the cytoplasm and nucleus, is expressed mainly in the vascular tissues in the roots and phloem companion cells in the leaves. In soils with lower Cd concentrations, the Cd content in the grains of the *lcd* mutant is approximately half that of the wild type. However, there is no significant difference in the Cd content within the nutritive organs of the *lcd* mutant compared with the wild type (Shimo et al, 2011). This raises the hypothesis that OsLCD may enhance Cd accumulation by activating other Cd transporters. Further research is essential to identify additional regulatory factors that control plant Cd detoxification and tolerance.

Molecular breeding of low-Cd crops

Cd accumulates in plants and animals with a biological half-life estimated at approximately 25–30 years (Genchi et al, 2020).

Various countries and regions have instituted differentiated regulatory thresholds for allowable concentrations of Cd in agricultural products. For instance, Chinese standards, as delineated in ‘Limits of Pollutants in Food’, cap the maximum level of Cd in rice at 0.1 mg/kg, whereas for legumes, the limit is 0.2 mg/kg. The Food and Drug Administration of the United States mandates that the Cd content in rice and

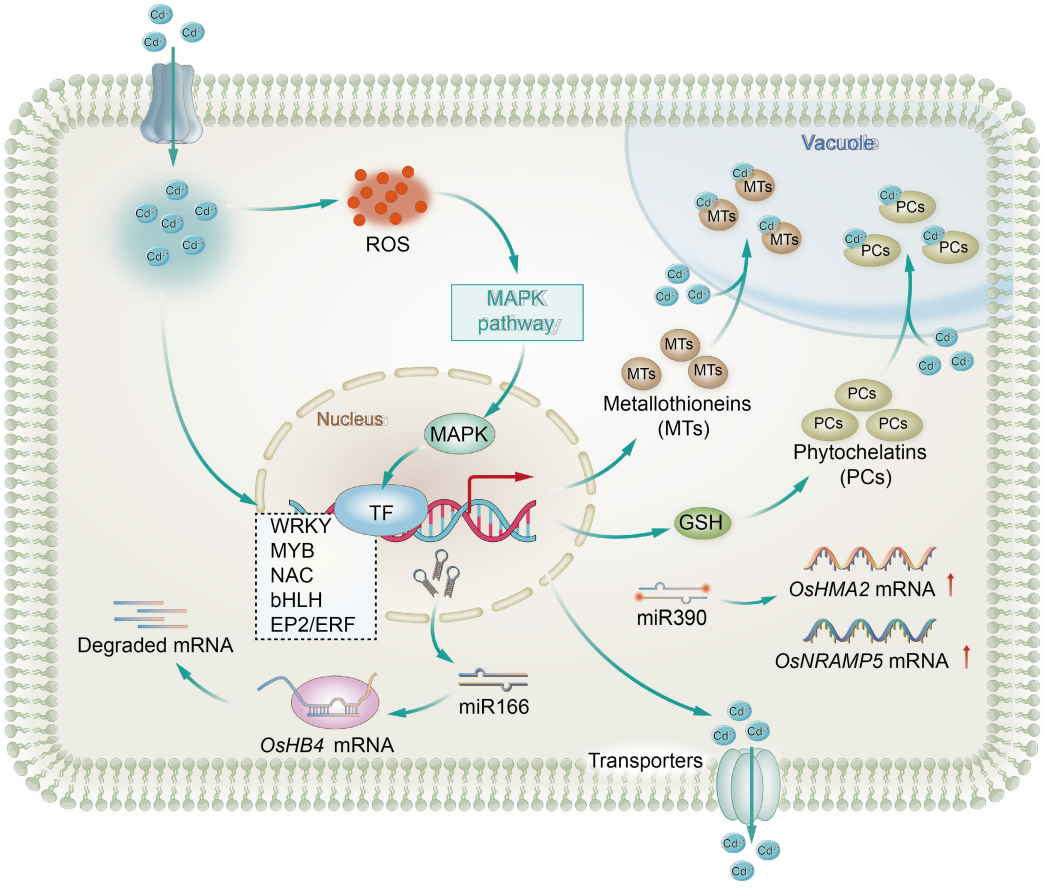

Fig. 1. Presumed transcriptional network associated with cadmium (Cd) signaling in rice.
Upon entry of Cd^{2+} (blue circles) into the cell, it leads to the accumulation of reactive oxygen species (ROS, red ellipsoids), activing the mitogen-activated protein kinase pathway (MAPK, green ellipsoids) that transmits the signal to the nucleus. This influences transcription factors (TF, blue ellipsoids), which, in turn, regulate the downstream expression of Cd transporters (aggregate of ellipsoids), metal-binding proteins (brown ellipsoids), and metal chelators (yellow ellipsoids). Additionally, miR390 increases the expression of *OsNRAMP5* and *OsHMA2*, enhancing Cd uptake and transport in rice, while miR166 degrades *OsHB4* mRNA, reducing *OsHB4* expression and thereby decreasing Cd accumulation and increasing Cd tolerance. GSH, Glutathione.

rice products should not surpass 0.1 mg/kg. The European Community has set the Cd content limit in wheat to a range of 0.1 to 0.2 mg/kg. Considering these regulatory frameworks, imperative measures are necessary to curb the toxicity and bioaccumulation of Cd in crop plants, with particular emphasis on edible tissues. Several strategies have been deployed to minimize the toxicity of Cd, which can be broadly categorized into: (1) the direct reduction of heavy metal content in the soil, which is further divided into physical, chemical, and biological methods; (2) the immobilization of bioavailable heavy metals in soil to reduce their uptake by crops; and (3) the optimization and genetic improvement of plant cultivars to diminish Cd bioaccumulation.

Molecular breeding is the generic term encompassing various cutting-edge breeding strategies such as molecular marker-assisted breeding (MAB), transgenic breeding, and genome editing technologies (Visarada et al, 2009; Ribaut et al, 2010; Hua et al, 2019). Molecular breeding, as a contemporary breeding technique, offers unique advantages through the precise enhancement of plant resistance to Cd, positioning it as the optimal choice for sustainable and safe agricultural production in heavy metal-contaminated fields.

MAB breeding

MAB leverages polymorphisms within DNA sequences as a foundation for genetic marker technology. This approach harnesses the precise identification and tracking of known QTLs or specific genes, thereby facilitating the selection of crops at the genetic level. By utilizing MAB, researchers can bypass the waiting period associated with plant growth and flowering to

ascertain phenotypic traits, thus considerably accelerating the breeding cycle (Jiang, 2013). Rice, endowed with comprehensive whole-genome genotypic data, high-density molecular markers, and well-established transformation techniques, has witnessed rapid advancements in MAB for low Cd cultivar development.

Specific loci within genes like *OsHMA3* and *OsNRAMP5* have been identified as significantly associated with cadmium accumulation. These loci can be developed into molecular markers for identifying Cd accumulation traits. Loss-of-function alleles of *OsNRAMP5*, such as *lcd1* (Cao et al, 2019), *lch3* (Wang et al, 2019), and *lcd-kmt1/2* (Ishikawa et al, 2012), have been associated with a significant reduction in the uptake of Cd in rice roots. High-throughput genomic sequencing of 1 143 *indica* accessions has facilitated the discovery of natural deletions in *OsNRAMP5* within the Luohong 3A and Luohong 4A rice varieties (Lv et al, 2020). Utilizing a 2 980 bp sequence (Tons) on rice chromosome 7 of the low Cd accumulating variety Luohong 4A, a molecular marker detection system for Tons was developed. This innovation enables the derivation of a novel low-Cd genetic material, named Tonys, derived from hybrid offspring of Luohong 4A (Chen et al, 2023). In rice, by hybridizing Cho-ko-koku with Akita 63, the variety Akita 110 was developed, specifically engineered for phytoremediation of Cd-contaminated soil. During the selection process in the F_5 generation of this crossbreed, the DNA marker based on *OsHMA3* was employed for genotypic analysis, facilitating the identification and selection of plants bearing the Cho-ko-koku genotype (Takahashi et al, 2016).

Within durum wheat, several molecular markers have been discerned for their linkage to loci governing Cd accumulation. Among these, OPC-20 is associated with the major locus *Cdu1* involved in Cd accumulation (Penner et al, 1995), and markers such as usw47 and Cad-5B (Salsman et al, 2018; Alsaleh et al, 2022), Ex_c1343_2570756 (Salsman et al, 2018) have been identified and utilized to enhance the selection process for low-Cd durum wheat varieties.

In maize, exploiting the inherent sequence variations within the *ZmHMA3* gene, four PCR-based molecular markers were developed spanning various maize lines. These markers effectively distinguished five haplotypes, linked to varying grain Cd contents within the genome-wide association study panel. The employment of these molecular markers is anticipated to contribute substantially to the breeding of maize cultivars with inherently lower grain Cd concentrations (Tang et al, 2021). In soybean, *GmHMA1a*, which shares a 49.8% similarity with *AtHMA3*, has been the focus of a derived cleaved amplified polymorphic sequence marker development for *GmHMA1a*. This marker has demonstrated a significant correlation with the Cd concentration in soybean seeds (Benitez et al, 2012).

However, the restricted pool of effective molecular markers remains a principal hindrance to the application of MAB in selecting low-Cd progenies in staple crops. With the rapid advancement of next-generation sequencing and RNA sequencing analysis techniques, there is potential to identify additional QTLs and molecular markers that regulate Cd absorption, transport, and accumulation within the grain.

Genome editing in crop breeding

As previously elucidated, a compendium of crucial genes regulating Cd transport and accumulation has been cloned and characterized in recent years. The advent of pioneering technologies such as RNA interference (RNAi) and the CRISPR/Cas9 genome editing system has furnished researchers with the capacity to precisely modulate these functional genes, thereby expediting phenotypic modification and enhancing the efficiency of crop breeding endeavors.

In *indica* rice, the deployment of RNAi to suppress the expression of the *OsPCS* gene within the grains led to the creation of three mutant lines (*ihpL1*, *ihpL2*, and *ihpL3*), exhibiting low-Cd accumulation. Analysis of these transgenic rice lines grown under heavy metal stress revealed a substantive reduction in Cd and arsenic (As) content in the harvested grains, approximately 51% and 35% lower, respectively, than in non-transgenic plants (Das et al, 2017). Moreover, the *OsCd1* genotype exhibits significant differentiation between *indica* and *japonica* rice. Specifically, the variant *OsCd1V449*, which is predominantly found in *japonica* rice, echoes a reduced capacity for Cd transport relative to its *indica* gene counterpart *OsCd1D449*. By integrating the low-Cd allele *OsCd1V449* into the *indica* cultivar 9311, the grain Cd accumulation was successfully reduced, suggesting the application value of *OsCd1V449* in a wider range of *indica* backgrounds (Yan et al, 2018).

Employing the CRISPR/Cas9 system, the metal transporter gene *OsNRAMP5* was effectively disrupted in the male sterile line 638S and the restorer line HZ of *indica* hybrid rice. This manipulation gave rise to novel low-Cd, non-transgenic *indica* rice lines.

Subsequent field trials in Cd-contaminated rice paddy environments demonstrated a consistent reduction in the Cd concentration in the grains of the *osnramp5* rice mutants, with levels persistently below 0.05 mg/kg. Notably, the *osnramp5* mutation does not have a significant impact on plant yield (Tang et al, 2017). Additionally, mutants of both *OsLCT1* and *OsNRAMP5* were generated using CRISPR/Cas9-mediated genome editing. Pot experiments revealed that the *lct1-1* mutants exhibit heightened sensitivity to increased soil Cd concentrations in contrast to the *osnramp5* mutants, suggesting that *lct1-1* may be suited for producing consumable rice grains in lightly contaminated paddy soils, while *osnramp5-7* could be optimal for application in soils with higher Cd pollution (Liu S M et al, 2019). Given that OsNRAMP5 is the primary transporter protein responsible for Mn and Cd uptake in rice, the knockout of *OsNRAMP5* might inadvertently affect the absorption of Mn, an essential micronutrient critical for plant growth and development. Potential Mn deficiency resulting from disrupted *OsNRAMP5* could inhibit growth and lead to reduced yields. When CRISPR/Cas9-based gene editing was utilized to knock out *OsNRAMP5* in *japonica* varieties Nanjing 46 and Huaidao 5, although Cd content in flag leaves and grains was significantly reduced in the *osnramp5* mutants, certain agronomic traits including plant height, seed-setting rate, and grain number per panicle were affected in the mutants, which ultimately caused a modest decrease in grain yield (Wang et al, 2019). Meanwhile, in the *japonica* cultivar Zhonghua 11, both the T-DNA insertion mutants and RNAi lines targeting *OsNRAMP5* displayed pronounced growth suppression, leading to a marked reduction in grain yield (Ishikawa et al, 2012, 2020). Therefore, in the process of breeding rice varieties with low Cd accumulation using *osnramp5* mutants, it is essential to monitor the uptake of Mn. Field trials may be necessary to ascertain the suitable ecological regions for cultivation. Mitigating Mn deficiency could potentially be achieved by inducing various mutation sites or employing different genetic backgrounds. Alternatively, the direct application of Mn fertilizers might alleviate Mn deficiency.

Conclusions

Exposure to Cd presents significant risks to various organisms, including human beings, stemming from its array of adverse effects. As such, elucidating the mechanisms of Cd absorption, transport, and accumulation in rice constitutes an essential endeavor in formulating efficacious measures aimed at diminishing Cd content in crops. Over the past two decades, significant progress has been achieved in identifying and characterizing mechanisms implicated in the mediation of Cd transport within rice (Fig. 2). These mechanisms affect both Cd transport and accumulation, providing insights into how environmental factors and genetic variables impact Cd levels in crops.

However, there are certain challenges when applying these genes for crop breeding. A particular concern is the limited number of genes that specifically regulate plant Cd uptake, which presents a significant bottleneck. Furthermore, most of these genes are also involved in the transport of other metallic elements as well. Modifying a single gene may concurrently perturb the uptake of other essential elements, thus adversely impacting plant growth and yield. For instance, *NRAMP5* plays a role in regulating the transport of both Mn and Cd, and *ZIP5*, *ZIP7*, and *ZIP9* are essential for Zn transport. Mutations in these genes have been shown to reduce cereal yields (Tan et al, 2019, 2020). Therefore, while characterizing and utilizing these functional genes, it is essential to further investigate their impact on the transport of other metallic elements. This approach aims to strike a balance between reducing Cd accumulation in crops and maintaining the equilibrium of other essential metallic elements, thereby ensuring the normal growth of crops.

Genome-editing technologies in crop breeding allow for precise modifications in specific genes on chromosomes, offering advantages of precision, efficiency, flexibility, and scalability over traditional breeding methods, such as the introgression of mutant alleles through hybridization. Significant progress has been made in genetically improving low-Cd rice cultivars using the CRISPR/Cas9 system. However, its practical application faces constraints owing to regulatory policies governing the use of genetically modified organisms (GMOs). It is worth noting that, in many countries or regions, crops edited using CRISPR/Cas9, when free of foreign DNA, do not fall under the GMO categorization. Consequently, this legal distinction can facilitate the integration of genome-edited crops into agricultural practices, provided they adhere to local legislative frameworks. Additionally, molecular breeding methodologies need not be seen as isolated approaches; rather, they can operate synergistically with other conventional and emerging breeding methods, thereby enriching the

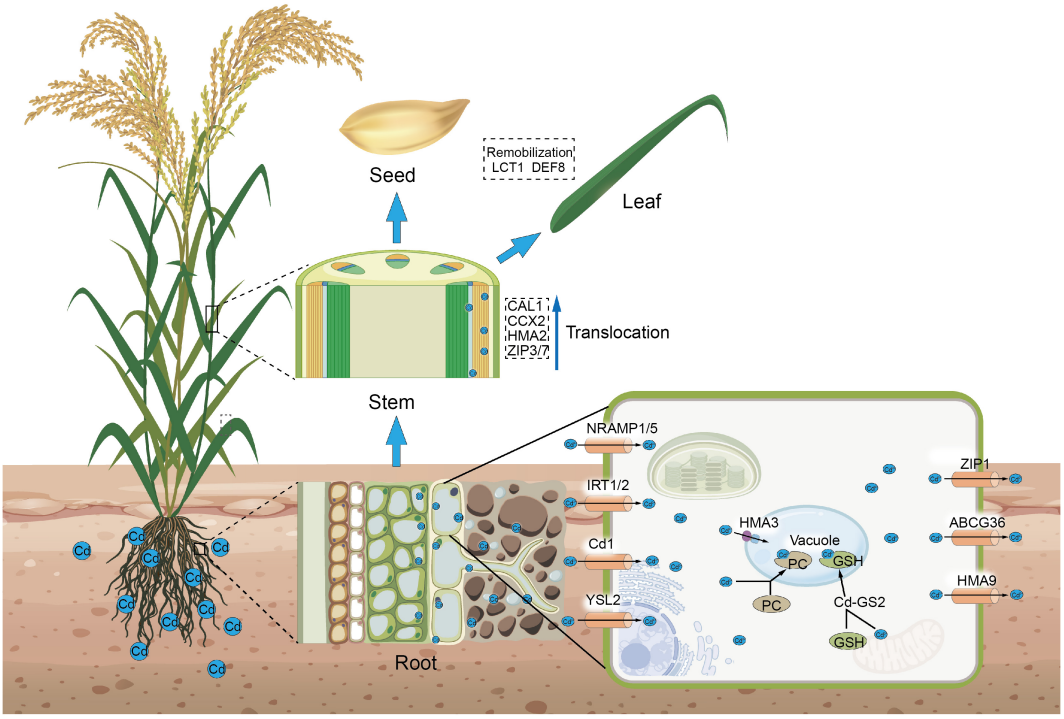

Fig. 2. Schematic diagram of cadmium (Cd) uptake, transport, and remobilization in rice.
The blue dots represent Cd and Cd^{2+}, while the cylindrical shapes on the cell membrane depict proteins that facilitate the entry and exit of Cd^{2+}. The ellipsoids, colored brown and green, represent the chelators of phosphatidylcholines (PCs) and glutathione (GSH), respectively. Proteins within the dashed rectangular structures are involved in the transport of Cd in the stem, as well as those participating in the mobilization of Cd in seeds and leaves.

toolbox available for the creation of Cd-resistant crop varieties. As both technological innovations and regulatory landscapes continue to evolve, molecular breeding offers a broader application perspective for of Cd-resistant crop breeding on a global scale.

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