



## Cadmium minimization in rice. A review

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# Cadmium minimization in rice. A review

Abin Sebastian · Majeti Narasimha Vara Prasad

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**Abstract** Cadmium (Cd) contamination of rice is found in areas irrigated by wastewater from mines. Cd contamination of rice fields can also result from the application of Cd-rich phosphate fertilizers. As a consequence, millions of tons of rice are discarded. In Asia, irrigated paddy-based cropping systems provide rice grains as food for about 2 billion people. A daily intake of 20–40 µg Cd from rice is reported in regions where rice is used as a food. Daily rice Cd intake leads to diseases such as bone mineralization. Hence, Cd minimization in rice is needed. This article reviews sustainable agriculture and molecular techniques that prevents Cd uptake in rice. Cadmium minimization can be done either by field remediation or change in plant functions. Organic farming decreases Cd uptake and remediates crop fields. Cd hyperaccumulator plants and Cd immobilizing microbes can be used for field remediation. Cd amount in rice can be controlled by gene families that code for putative transition metal transporters or metal chaperones and quantitative trait loci (QTL). Generation of Cd excluder rice is possible by transgenics.

**Keywords** Biogeochemistry · Ecophysiology · Phytotechniques · Cadmium transporters · Metal chelators · Cadmium responsive genes · Cadmium excluder rice

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## 1 Introduction

Cadmium (Cd) is a toxic trace element belongs to group II B of periodic table of elements. Cd accumulation pose serious health implications in human such as anemia, hypertension, cancer, cardiac failure, cerebrovascular infarction, emphysema, proteinuria, serious damage in lungs, renal dysfunction, cataract formation in eyes, and osteoporosis (Godt 2006; Satarug et al. 2003). Major route of Cd uptake in human is through daily intake of food stuffs that contain Cd (Satarug et al. 2003; Wani et al. 2007). Average daily intake of Cd is considered as 30 µg that varies between populations (Figuerola 2008). Usage of Cd in industrial applications such as anticorrosive agents for aircraft, stabilizer in polyvinyl chloride (PVC) products, color pigments, neutron-absorber in nuclear power plants, and fabrication of nickel–cadmium batteries has resulted in the increase in demand of Cd (Cotuk et al. 2010; Nriagu and Pacyna 1998).

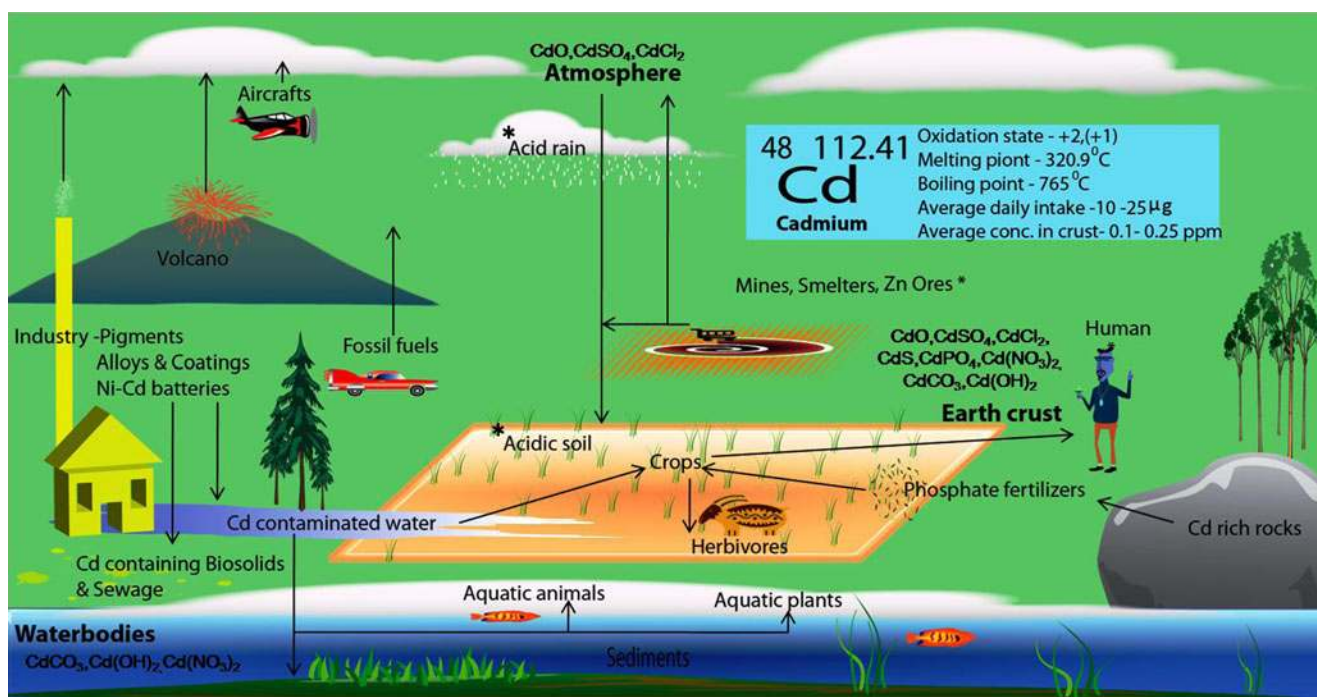
### 1.1 Biogeochemical cycle of cadmium

Biogeochemical cycle of Cd indicates that the metal is released into the environment as an anthropogenic pollutant while emission from natural sources such as volcanic activities, weathering of rocks, and soil erosion are locally

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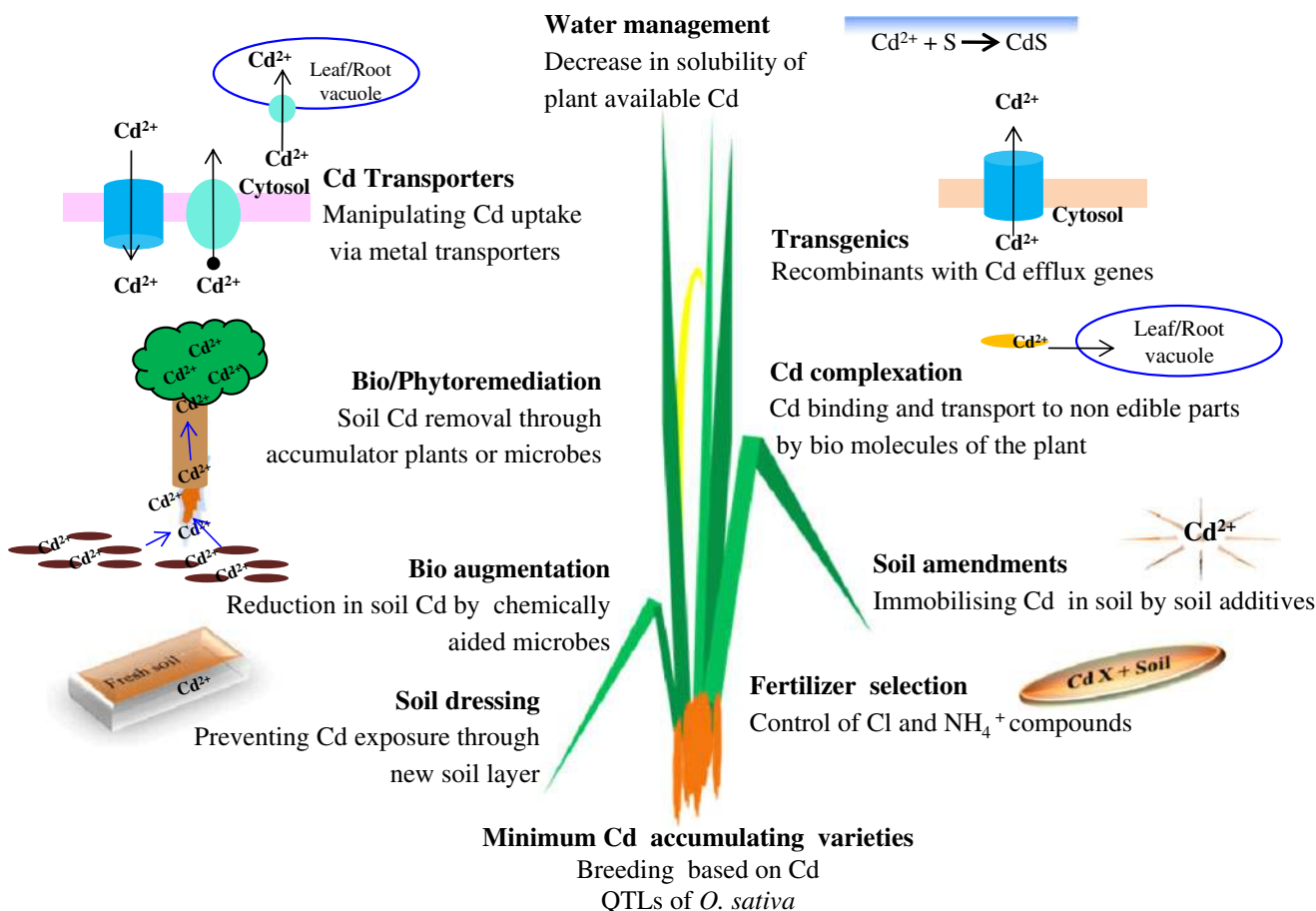
limited (Adamu and Nganje 2010; WHO 1992) (Fig. 1). Nitrogen containing Cd compounds such as cadmium nitrate are also found in the environment as a result of reduction reaction of nitrate with metallic Cd. Studies on contribution of automobiles to environmental Cd release found that plants accumulate Cd near traffic where source of Cd is zinc (Zn) containing additives in motor oil and Zn compounds use in vulcanization of rubber tires (Aslan et al. 2011; Lagerwerff and Specht 1970). Burning of coal, smelters of nickel–Cd batteries, and electroplating are also found to be routes of Cd emission into the atmosphere that could transfer Cd into soil as well as vegetation cover through wet or dry deposition (Schoeters et al. 2006). Runoff from waste piles of mines especially Cd, Zn, and Pb are considered among the major routes of Cd contamination of rice fields (Sandalio et al. 2001). Other routes of Cd entry into paddy soil are agricultural practices such as application Cd-containing fungicides, super phosphate fertilizers, and Cd-containing sewage sludge application in the rice field (WHO 1992; Wang et al. 2009). Studies on provisional tolerable weekly intake (PTWI) indicate that major food sources that contribute Cd to human diet are rice, wheat, starchy roots/tubers, mollusks, and shellfish (Baize et al. 2009; Figueroa 2008; Flick et al. 1971). Application of fertilizers,

genotype of crop use for cultivation, crop rotation, and dietary deficiencies are also factors which influence human exposure to Cd (Figueroa 2008). Among a wide range of Cd polluting sources, repeated application of Cd-containing phosphate fertilizers and irrigation using Cd-contaminated water releasing from mines are causing massive contamination of Cd in rice fields (Lugon-Moulin et al. 2006; Sandalio et al. 2001; Yang et al. 2006). In a well-documented incident of Cd poisoning, itai-itai disease spread in Toyama prefecture in Japan was through daily intake of rice grains which were harvested from areas irrigated with Cd-contaminated waste water release of mines (Kobayashi 1978). A recent report from China covers Cd contamination of 1700,000 ha of farmland in northern Guangdong province of China (Yang et al. 2006). Since rice grain contributes a diet rich in vitamins, minerals, and complex carbohydrates for half the world population, there is persistent demand for Cd minimization in rice. The search for reducing Cd in rice opens the need to explore scientific approaches to minimize Cd in rice plants and hence reduction in the amount of Cd in the grain (Fig. 2). Application of Cd minimization strategies essentially requires a better understanding of ecophysiology of Cd uptake that links Cd in soil with rice plant, feasible field remediation measures that prevent Cd uptake into rice,



**Fig. 1** Biogeochemical cycle of cadmium. Cadmium is a transition metal which is released into environment as a technogenic pollutant. It enters the atmosphere either by anthropogenic activities or natural sources like volcanoes. Entry of Cd from polluting source to the earth's crust can be direct or indirect by wet and dry precipitation of atmospheric Cd compounds. Cd contamination of water bodies occurs mainly via release of Cd-rich water from various industrial sources as well as mine leachate. Cd occurs preferentially at different chemical

forms in biosphere as indicated in the figure. Phosphate fertilizers produced from rock phosphate containing Cd are an inevitable source of Cd in rice field. The entry of Cd into humans is through the food chain while occupational exposure is negligible. \*Acid rain as well as acidic soils have an influence on Cd uptake in vegetation by lowering soil pH which enhances Cd uptake by plants. Irrigation using water released from mines and smelters is the major route of massive Cd contamination in rice fields



**Fig. 2** Strategies for cadmium minimization in rice. Water management keeps the reducing condition in field which allows Cd precipitation. Phytoremediation, soil amendments, bioaugmentation, and soil dressing are field remediation steps which are applicable prior to rice cultivation in the field. Manipulation of Cd-responsive metal transporters and use of genes of

such metal transporters allow exclusion of Cd from cytosol of roots as well as targeting Cd to vacuole in non-edible parts. Cd transport to the non-edible part of the plant can be accelerated through control of Cd complexation aided with metal chelators. Plant breeding focuses on quantitative trait loci (QTL) of Cd and breeding of rice varieties with lower Cd accumulation capacity

and the factors affecting Cd uptake and partitioning in rice plants which are useful to generate rice plants resistant to Cd. The following sections sequentially describe strategies to minimize Cd in rice.

## 2 Ecophysiology of cadmium uptake

Cadmium uptake by rice plant depends on ecophysiological components. Ecophysiological components belong to physical characters of field and morphological features of the plant. Physical components that influence Cd uptake are redox potential of the soil, soil pH, essential trace element status, and organic matter content in the soil (Jung 2008; Matsi et al. 2007; Singh and Myhr 1998). These ecophysiological components promise various field management practices that can be utilized for minimizing Cd in rice (Fig. 3). Rice field development is characterized by dependence on field management irrespective of soil conditions prior to cultivation. Field management practices often lead

to specific soil horizon in lowland rice fields having four parts such as a top layer of water that forms plankton as well as microbial habitats, a zone with highly dynamic oxidation states, puddled layer characterized by lack of molecular oxygen, and plough pan where steady reducing state is maintained (Kögel-Knabner et al. 2010; McDonald et al. 2006). This kind of field management often leads to loss of clay particles during heavy rain runoff which creates disturbance in the soil profile (Garg et al. 2000; Gong 1983). A noticeable feature of such a rice field is redox dynamics that significantly affect uptake of Cd by rice (Cattani et al. 2008). Most of the growth period of rice is under anoxic condition whereas harvesting period is characterized by oxygenic condition which leads to an increase in redox potential of soil during which compounds of iron (Fe) get oxidized (Magneschi and Perata 2009). Thus, puddled layer and the plough pan make a soil horizon characterized with reducing power formed through segregation of Fe and manganese (Mn) matrix, which could precipitate soluble fraction of Cd in soil (So and Ringrose-Voase 2000).





**Fig. 3** Field managements for cadmium minimization in rice. **a** Essential water supply in the field ensures redox potential in the field and reduces Cd entry in to the rice by favoring Cd precipitation reactions. **b** Foliar micronutrient application especially at the time of tillering as well as grain maturation reduces the chance of nutrient deficiency adaptations such as release of phytosiderophores that favors Cd uptake. **c** Application of wood ash or biochar improves soil properties and replenishes

micronutrients in the field. This approach increases Cd-holding capacity of soil. Burning of wood in the field also has advantages in terms of destruction of various pathogens and spores of pathogen. **d** Crop rotation with legumes not only removes Cd in the field but also enhances nitrogen content and hence reduces requirement of ammonium-based nitrogen fertilizers decreasing soil pH

## 2.1 Soil redox potential adjustments

Soil redox potential adjustments help to control Cd solubility. Temporal and spatial changes in redox potential affect soil organic matter constituents and mineral constituents (Gallardo 2003). Cd solubility was found to decrease during reduction of soil suspension likely due to sorption to aluminum, Fe, and/or Mn oxyhydroxides or precipitation of CdS or  $\text{CdCO}_3$  (Arao et al. 2009; Chlopecka 1996; Sarwar et al. 2010). Hence, it is important to have a flooding period before heading which significantly reduces Cd uptake into rice. A 16-fold reduction of Cd in rice was observed in a field trial where 8 days before heading period flooding was trialed (Arao et al. 2009). Formation of water-insoluble cadmium sulfide (CdS) was attributed as the reason for decrease in Cd uptake in this study. On the other hand, when soil is drained, Cd forms cadmium sulfate ( $\text{CdSO}_4$ ) which is soluble in water and causes higher uptake of Cd into rice. Chloride level in water is another concern which favors Cd uptake through the formation of exchangeable cadmium chloride ( $\text{CdCl}_2$ ) complex (Smolders and McLaughlin 1996). Chlorosalinity also reduces soil sorption of Cd and increases Cd in the solution which leads to an increase in mobility and adsorption of Cd to roots (Liu et al. 2010; López-Chuken et al. 2010). Hence, chlorination of irrigation water must be under tight control to reduce Cd in rice. It is

noteworthy that control of application of potassium fertilizers based on potassium chloride (KCl) that creates an increase in uptake of Cd by rice plants.

## 2.2 Soil pH and nutrient management

Soil pH and nutrient management allows lowering of Cd availability. Soil pH is featured as a key factor that controls the amount of exchangeable Cd in soil for plant uptake. Acidic soil was found to have more plant-exchangeable Cd (Appel et al. 2008; Sauvé et al. 2000). Cd adsorptive capacity of soils was reported to increase by a factor of three for one pH unit between pH 4.0 and 7.7 (Christensen 1984). Soil pH changes in paddy fields are also uneven (Kögel-Knabner et al. 2010). During the early stage of crop growth, soil pH is neutral and turns into alkaline during the course of harvest. Consumption of proton often leads to an increase in soil pH of acidic soil under aerobic condition whereas flooding results in a decrease in soil pH due to  $\text{CO}_2$  production in rice fields (Kögel-Knabner et al. 2010). These point roles of increase in soil respiration that leads to an enhanced Cd uptake by rice. A high soil pH value usually favors sorption of Cd in soil which decreases partitioning of metal for plant uptake (Wang et al. 2006). Counter effect of industrial environmental pollution to soil pH and Cd uptake in rice plants could also be exemplified from the report that

a 10-fold increase in plant-available Cd was a result of acid rain (Hutton et al. 1987).

Cadmium-holding capacity of top soil is usually higher, and the addition of biosolids often increases Cd-holding capacity as well as essential micronutrients which retard Cd uptake by plants (Hamon et al. 1998; Rate et al. 2004). Transfer of Cd from soil to plant also depends on the level of micronutrients such as Zn, calcium (Ca), and magnesium (Mg) (Chaney et al. 2004; Haouari et al. 2012). Soil deficient in mineral nutrients has more exchangeable Cd and hence higher likelihood of uptake of Cd by plants. Phosphorous in the soil precipitates Cd by forming insoluble phosphate complexes (Street et al. 1978). Hence, if the field is deficient in phosphorous, application of diphosphate fertilizer devoid of Cd helps to reduce plant-available Cd in soil. It is better to use diphosphate than trisuperphosphate to reduce Cd, which causes efficient sorption of Cd into the soil (McLaughlin et al. 1995). In nutrient-rich soil, phosphate has disadvantages in that it decreases Zn uptake which may lead to an enhancement in uptake of Cd by plants (Jiao et al. 2004; Root et al. 1973). It is reported that the presence of essential nutrients such as Ca, Zn, Mn, Fe, and copper (Cu) was found to inhibit Cd uptake in plants (Cataldo et al. 1983; Sarwar et al. 2010). But these observations are controversial since nutrient uptake is controlled by multiple factors which may create paradoxical results. Agricultural practices for increasing yield through ammonium-based nitrogen fertilizers also lead to higher Cd uptake by rice because of soil acidification (Casova et al. 2009; Eriksson 1990). Hence, control of ammonium fertilizer must be done to avoid soil acidification which helps to prevent release of plant-available Cd in soil.

### 2.3 Soil organic matter management

Soil organic matter management help to prevent dynamics of soil Cd. Application of organic fertilizers such as farmyard manure, azolla manure, etc. improve the soil physical structure and could be an alternative to synthetic fertilizers. It is reported that abiotic and biotic factors such as soil pH, anoxia, iron oxyhydroxides, clay minerals, aluminum hydroxides, and microbial communities play a significant role in determining the amount of organic matter especially soluble organic matter in natural paddy soils (Kögel-Knabner et al. 2010; Oyewole 2012). An increase in organic matter often affects soil Cd by adsorption of metal resulting in a decrease in plant-available Cd while weathering of organics in soil has a reverse effect (Brams and Anthony 1983). The presence of dissolved organic matter often increases the chance of reducing events. Hence, reducing condition that prevails after flooding through the incorporation of organic matter increases the chance of binding of Cd to organic matter and reduces plant-available Cd in soil

(Sauve et al. 2003). It is also observed that at higher soil pH, organic matter forms soluble Cd complexes which make Cd unavailable for ion exchange process with roots (Adriano et al. 2004). Another factor of concern is removal of crop residues after harvest where residues after decomposition favor an increase in soil Cd level. Crop rotation with legume also has a significant role in determining plant-available Cd level of the field. Soil surrounding legume rhizosphere is acidified through nitrogen fixation process and favors uptake of Cd by plant (Rao et al. 2002). Therefore, the growth of legume in the field helps to mobilize Cd and legumes remove Cd from the field by metal accumulation process. Mycorrhiza colonization was also reported to reduce uptake of Cd by creating a barrier before Cd entry into roots (Khan et al. 2000). Scope and application of mycorrhizae needs to be explored since rice plants do not have mycorrhizal association.

### 2.4 Morphology of rice and cadmium uptake

Morpho-physiological features of rice are in the direction of favoring uptake of Cd. For example, rice being a monocot with fibrous root system that increases surface area for mineral absorption is favoring the chance of uptake of Cd (Coudert et al. 2010). Chelating agents such as organic acid from rhizospheric microbes and phytosiderophores from rice plants are favoring Cd uptake in rice (Romheld 1991). Solubilization of Cd by a cell-wall-mediated process is also described as a factor that increases the chance of Cd uptake by root cells (Clemens et al. 2002). A noticeable feature of rice is that plaque formed from iron and manganese which precipitate at root apoplast as a result of development of aerenchyma tissue. This tissue has higher redox potential during the course of submergence and makes Cd unavailable for translocation into shoot (Lui et al. 2008). It is noteworthy that the majority of Cd found in root resides at root apoplast and bind with cell wall polysaccharides (Zhang et al. 2009). Low diffusion coefficients of Cd in aqueous solution indicate that uptake of Cd into root depends on the transpiration pull and also point out the importance of water management in controlling Cd uptake (Hagemeyer et al. 1986; Lux et al. 2011).

### 2.5 Physiological biomarkers of cadmium

Physiological functions in plants are affected by Cd (Benavides et al. 2005; Hasan et al. 2009; Mediouni et al. 2006; Prasad 1995). Many of biotic as well as abiotic stress responses are also observed in Cd-induced stress (Gill and Tuteja 2011). One of the immediate responses of Cd toxicity is loss of plant pigments (Vassilev and Lidon 2011). Other processes such as photosynthesis, water use efficiency, and nutrient assimilation are also reported to be affected by Cd



(Nazar et al. 2012; Tóth et al. 2012; Wahid et al. 2008). Among biochemical functioning; antioxidant enzyme pathways are extensively studied (Gill and Tuteja 2011; Shah et al. 2001; Wang et al. 2011). The influence of Cd on synthesis of cellular proteins such as rubisco, photosystem II, etc. points out the influence of Cd on sugar metabolism which ultimately affects overall growth of the plant because of the indirect relation of sugar metabolism with other cellular pathways such as aminoacid synthesis, fatty acid synthesis, etc. Thus, characterization of physiological functions in the presence of Cd helps to screen rice varieties for Cd tolerance as well as indicates the level of Cd contamination in the field. Since many physiological functions are under the control of quantitative loci, physiological monitoring aids to develop Cd-resistant varieties and approaches that are being taken for improving crop yield have an influence on Cd tolerance. Thus, crop improvement programs in rice should be monitored for Cd resistance too. In summary, ecophysiology of Cd uptake postulates field management practices such as artificial submergence and drainage, organic manuring, liming, and phosphate fertilization which are efficient to prevent Cd uptake. These practices are also found to affect redox status of the soil and hence help to control amount of exchangeable Cd in the soil. Crop morphology and physiological functions need to be matched for a low Cd accumulating trait.

### 3 Phytotechniques and sustainable field remediation

Removal of Cd or change of mobility Cd in soil is essential to prevent Cd entry into rice. Application of soil amendments, phytoremediation, and bioremediation are in use for

large-scale field remediation programs (Chen et al. 2000; Lovley and Coates 1997). Key process in these methods are binding of Cd with amendment that results in the reduction of plant-available Cd, removal of Cd from field using hyperaccumulator plants, and biosorption of metals or enzymatically catalyzed changes in redox state of metal by microbes respectively. An advantage of phytotechniques such as the use of organic amendments is that these amendments are ecofriendly as well as cost effective for use in large-scale field remediation (Fig. 4).

#### 3.1 Soil amendments

Soil amendments are soil additives able to improve the physical characters of soil such as water-holding capacity, water permeability, water infiltration, aeration, and structure of soil (Madejón et al. 2006). The list of amendments that are reported to reduce Cd uptake in plants is given in Table 1. Most of the amendments work by reducing plant-available Cd through phenomenon of sorption, adsorption, precipitation, immobilization, and stabilization of metal in the soil (Adriano et al. 2004; Wuana and Okieimen 2011). In case of organic amendments, periodic change of amended material must be done in order to back up metal-holding capacity after decomposition of the older one. Amendments mainly affect partitioning of Cd between solid and liquid phases of the soil. Sorption properties of organic amendments were found to have increased Cd binding properties of soil around 30 times than mineral soils (Save et al. 2003). Organic amendments form a coating over a particulate matter especially on surface and subsurface layers of soil and could act as metal binder. These amendments are characterized by the presence of lignin, cellulose, tannins, and

**Fig. 4** Phytotech approaches for rice cultivation to minimize Cd accumulation. **a** Rhizofiltration of pollutants in water using *Eichhornia crassipes* (water hyacinth) prior to irrigation of crops removes heavy metals such as cadmium. **b, c** Cultivation of aquatic macrophytes such as *Azolla pinnata* enriches nitrogen fertilization and removes pollutants. **d** Farmyard manure (FYM) pit with cow dung covered by rice straw, a traditional practice for long-term storage of FYM. Application of FYM immobilizes Cd in soil with aid of humic/fulvic acids. Therefore, FYM usage as fertilizer prevents Cd loading in rice grain



**Table 1** Amendments used for remediation of cadmium-polluted sites

Amendments	Reference
Paper mill sludge	Battaglia et al. 2003
Fe/Mn oxide	Livera et al. 2011
Charcoal	Wang et al. 2010
Bentonite	Karapinar and Donat 2009; Fernández-Nava et al. 2011
Perlite	Mathialagan and Viraraghavan 2002
Beringite	Adriano et al. 2004; Boisson et al. 1998
Clay minerals	Shirvani et al. 2007
Limestone	Basta and McGowen 2004
Mineral rock phosphate	Basta and McGowen 2004
Humus	Ok et al. 2011
Silica slag	Deja 2002
Lime	Ok et al. 2011
Gypsum	Poulsen and Dudas 1998
Hydroxyapatite	Boisson et al. 1999
Alkaline organic treatment	Bolan et al. 2003
Silicon	Kirkham 2006
Dolomite	Keller et al. 2005
Phosphate	Macaskie et al. 1987
K <sub>2</sub> HPO <sub>4</sub>	Adriano et al. 2004
KH <sub>2</sub> PO <sub>4</sub>	Adriano et al. 2004
Poultry manure	Han et al. 2008
Vermicompost	Pereira and Arruda 2003
Compost	McLaughlin et al. 1995
Peat	Ma and Tobin 2003
Leaf litter	Adriano et al. 2004
Sewage sludge	Mahler et al. 1978; Baize 2008
Cattle manure	del Castillo et al. 1993
Elemental sulfur	Livera et al. 2011
Zeolite	Chlopecka and Adriano 1997
Palygorskite	Wang et al. 2007

carbonates that increase natural capability of soil to retain heavy metals (Berg 2000). An organic amendment treatment leads to an increase in dissolved organic carbon (DOC) in soil. Formations of stable soluble DOC–metal complexes have been reported to reduce readily available metal for plant uptake from soil (Han et al. 2001). The presence of metal complexing agents in dissolved organic amendments such as humic acids and fulvic acids increases efficiency of organic amendments to absorb metal. These polyelectrolytes are characterized by the abundance of carboxyl and hydroxyl groups that bind with Cd (Lee et al. 1993). Application of potential organic amendments such as seed powder of weeds also could act as donor of functional groups where functional groups such as carboxyl react with Cd and precipitate Cd in soil (Jayaram and Prasad 2009). Utilization of weeds and economically important plants such as bamboo

which have potential to produce organic amendments for large-scale field remediation via utilization powder as well as charcoal prepared from plant parts that biosorb metals help sustainable field remediation (Lalhruiatluanga et al. 2010). Application of organic amendments in alkaline form also enhances carbon buffering of soil (Sloan and Basta 1995). Such treatments have the advantage that in addition to formation of Cd–carbonate precipitates and complexes, an increase of soil pH leads to sorption of Cd in soil. Exploitation of sewage as an organic amendment must be under monitoring because of the chance of Cd contamination in these materials (Moreno et al. 1999). Application of vermicompost, which appears to be ideal for Cd immobilization, is questionable where changes in soil structure upon addition of compost to soil such as increase in soil porosity enhance root growth (Sebastian and Prasad 2013). Enhanced root growth leads to Cd accumulation in rice plants by enhancing contact of root with Cd. Another drawback of organic amendments is the degradation of raw materials and hence the need for periodic addition of the materials used for immobilizing metal in soil.

Synthetic compounds are more efficient compared to natural organic amendments because of purity and the possibility of modifying them for a better metal immobilization practice. Calcium and ammonium phosphate crop fertilizers are reported to have potential to serve as chemical immobilizers of heavy metals (Basta and McGowen 2004). Thus, fertilizer combinations having phosphorous as inorganic amendment assure immobilization of Cd in soil. The presence of sulfur is also important which was found to cope with Cd uptake under reducing conditions (Mendoza-Cózatl et al. 2003). The nitrification process during the course of ammonium fertilizer application is also reported to influence Cd immobilization process. An increase in Cd accumulation is found during the nitrification process upon diammonium phosphate treatments which have potential to immobilize Cd in soil (Levi-Minzi and Petruzzelli 1984; Pierzynski and Schwab 1993). Clay materials such as montmorillonite, palygorskite, kaolinite, bentonite, zeolites, sepiolite, perlite, etc. are another promising category of metal-adsorbing amendments which have been shown to have high adsorption capacity due to high specific surface area, high cation exchange capacity, high Brönsted and Lewis acidities, and flexible surface change against pH (Lin and Juang 2002; Su-Hsia and Reuy-Shin 2002). Drawbacks of some of these materials are formation of stable colloidal suspension upon infiltration by water and hence the decrease in permeability of the soil. This drawback could be overcome by pretreatments such as granulation (Fernández-Nava et al. 2011). Activated carbon is also a promising adsorbent for Cd in soil while cost effectiveness is a drawback. Calcium carbonate is widely used to increase pH and reported to decrease metal uptake by crops (Knox et al. 2001). Requirement of a



large quantity of lime that must be repeated and shift in soil pH that affects essential nutrient uptake need to be considered during such lime treatments. Even though amendments are used widely to remediate field, the extent to which these materials can suppress metal uptake is debatable since they show variation in metal binding due to changes in soil properties.

Apart from amendments, physical methods in field management for Cd removal include methods such as soil dressing, soil washing, vitrification, chemical reduction, and electrokinetics (Bolan et al. 2013; Dermont et al. 2008). Thermal treatment, precipitation, sedimentation, ion exchange, reverse osmosis, and microfiltration are also applied in areas where large-scale contamination occurs. These methods are economically not feasible and recommended when contamination is at a higher extent. Washing of soil is usually performed with metal chelators such as ethylenediaminetetraacetic acid, triammonium bromide, calcium chloride, citric acid, hydrochloric acid, nitriloacetic acid, and other anionic biosurfactants (Huang et al. 1997).

### 3.2 Phytoremediation

Phytoremediation using hyperaccumulator plants are utilized to remove metal contaminants from soil (Prasad 2004). It is documented that introduction of metal-tolerant wild plants to metalliferous soils and genetic engineering of plants for enhanced synthesis for exudation of natural chelators into the rhizosphere are practical solutions to reduce exorbitant costs (Prasad and Freitas 1999). Selection of plant is critical in phytoremediation since surface area of root to soil contact plays a crucial role in metal accumulation. It is found that natural metal hyperaccumulator phenotypes are efficient in phytoremediation (Chaney et al. 1997; Prasad and Freitas 2002). Plants that belong to families such as Betulaceae, Brassicaceae, Carophyllaceae, Fabaceae, Fagaceae, Plumbaginaceae, and Poaceae are reported to accumulate toxic heavy metals where majority of them belong to Brassicaceae (Prasad and Freitas 1999a; 2002b). Fast growth rate and high biomass production rate are reported as key features of plants that must be taken into account during selection of a plant for phytoextraction (Pilon-Smits 2005; Schmidt 2003; Tang et al. 2003). Naturally growing grasses and weeds such as *Cyperus rotundus*, *Cyperus kylinga*, *Marselia quadrifolia*, and *Ludwigia parviflora* are reported to grow well in metal-contaminated sites and are useful in remediation of wetland rice fields (Sundaramoorthy et al. 2010). Plants such as *Pteris vittata* and *Thlaspi caeruliensis* that accumulate heavy metals are ideal choices for phytoextraction of Cd in upland rice fields (Ma et al. 2001; Milner and Kochian 2008; Zhao et al. 2003). Indian mustard, corn, sunflower, and sorghum are also reported to be effective in the

remediation of metal-contaminated fields while the extent to which they can extract Cd from conventional rice fields needs to be explored (Jadia and Fulekar 2008). Remediation of fields using oil-producing crops such as sunflower have the advantage that they not only remediate field but also provide an opportunity to generate by-products such as biofuels that add up to the economy. An approach of rotating crop plants with hyperaccumulators is also ideal to prevent Cd contamination of rice. Removal of water-soluble Cd from lowland fields could be possible through phytoremediation using aquatic macrophytes such as *Eichhornia crassipes*, *Lemna minor*, and *Pistia stratiotes* which are proven efficient in rhizofiltration (Karkhanis et al. 2005). It is also found that growth of aquatic plants like *Elatine hexandra*, *Althenia filiformis*, and *Monita rivularis* in flooded rice fields potentially removes Cd from the soil (Robinson et al. 2001). Application of algal biomass in the flooded plain to remove Cd was also found to be effective 3-fold compared to normal field conditions (Reniger 1977). Successive cropping and harvesting that reduces levels of contaminants in the soil is another approach where the removal of crop residues ensures periodic removal of metal contaminant (Vandenhove et al. 2001).

Phytoremediation accompanied by metal chelating molecules or symbiotic association such as mycorrhiza is another important tool in the process of phytoremediation (Prasad et al. 2010). Precipitation of Cd as polyphosphate granules in the soil, adsorption of Cd to chitin in fungal cell walls, and chelation of Cd inside fungus body are the chief characteristics of mycorrhiza-assisted soil cleanup (Joschim et al. 2009). The symbiotic interaction with hyphal network functionally extends root system and hence plants in symbiosis with mycorrhiza have more potential to take up metal from soil along with increase in water use efficiency and mineral uptake (Upadhyaya et al. 2010). Mycorrhiza association enhances essential mineral uptake especially phosphorous which has the ability to precipitate Cd. Thus, mycorrhiza restricts entry of Cd into roots. Increase of biomass through mycorrhiza association also leads to tissue dilution effect of Cd. This will reduce the amount of Cd which is able to reach the rice grain during grain maturation period (Wright et al. 1998). The potential of mycorrhiza to act as a filtration barrier against transfer of heavy metals to plants opens a scope of research to induce mycorrhiza association in rice plants.

### 3.3 Bioremediation

Application of microbial bioremediation is limited with regard to Cd minimization because Cd is not biodegradable. On the other hand, microbial immobilization of Cd and bioconversion of Cd complexes that limits metal availability to plant-assisted phytoextraction are promising to remediate Cd-contaminated rice fields. Microbial remediation of metal-

contaminated sites depends on factors such as binding of Cd to cell walls, metal concentration, pH, calcium concentration, ionic strength, and methylation of Cd (Christofi and Ivshina 2002; Vig et al. 2003). The carboxyl, phosphate, sulfhydryl, or hydroxyl functional groups acts as Cd adsorption sites on bacterial cell walls. This property makes microbial biomass to immobilize Cd in the field. Bacterial sorption of Cd was reported to be pronounced in sandy soil than in clay soil (Vig et al. 2003). It is reported that immobilized biosurfactant-producing bacteria *Bacillus subtilis* Tp8 and *Pseudomonas fluorescens* G9 remove Cd effectively from soil (Sarin and Sarin 2010). Sulfate-reducing bacteria which precipitate metal by forming metal sulfide are helpful in decreasing plant-available Cd. Application of alginate for soil inoculation of microbes is also promising to reduce exchangeable Cd. Renewal of alginate beads must be done in such applications because mineralization of alginate will lead to release of bound Cd (Jézéque and Lebeau 2008).

Siderophore-producing bacteria are gaining attention to remove metals from contaminated sites because of higher efficiency to mobilize metal in the soil (Rajkumar et al. 2010). Bioaugmentation is another fast-growing phytotechnology that use microbes to enhance metal availability and mobility. It is reported that *Pseudomonas fluorescens* Pf 27, *Variovorax paradoxus*, *Rhodococcus* sp., and *Flavobacterium* sp. increase water-soluble and exchangeable Cd content in contaminated soil. This in turn enhanced both plant biomass and uptake of Cd of in *Brassica juncea* (Belimov et al. 2005; Fuloria et al. 2009). Similar observations were also found in case of soybean inoculated with *Pseudomonas putida* 62 BN and *Pseudomonas monteilli* 97AN as well as canola inoculated with *Pseudomonas tolaasii* ACC23, *Pseudomonas fluorescens* ACC9, *Alcaligenes* sp. ZN4, and *Mycobacterium* sp. strains (Dell'Amico et al. 2008; Rani et al. 2009). Application of microbial fauna in rice field must be after field monitoring since rice plant is susceptible to various microbial pathogen attacks.

#### 4 Molecular perspectives in cadmium minimization

Apart from field-based techniques, modifications in rice crop which restricts Cd uptake ensure food safety. This approach also reduces economic losses due to field remediation programs especially in fields which are moderately polluted with Cd. It is reported that Cd uptake and allocation in various plant tissues are under control of genome (Fässler et al. 2011; Gallego et al. 2012). Cd tolerance in plants is also under control of genes which function by mediating metal chelator synthesis or exclusion of Cd through metal transporter mediated process (Clemens 2002; Hall and Williams 2003). Manipulation of these genes promises site-specific partitioning of Cd in plant tissues and hence

controls the amount of Cd which may accumulate in the grain. Difference in occurrence of Cd controlling trait observed among rice varieties also postulates the scope of genomic level of control for Cd minimization in rice grains (Yan et al. 2010). Occurrence of Cd-dependent quantitative trait locus (QTL) allows screening of rice varieties that are able to resist Cd uptake or accumulate Cd. Besides these, the search for genome via high throughput methods allows to find out Cd-responsive components in rice genome that help to develop rice plants with minimum uptake of Cd.

##### 4.1 Cadmium transporters and metal chelators

Cadmium transporters and metal chelators act as checkpoints in Cd uptake. Cd, being a non-essential element for plant growth, limits existence of a Cd-specific transporter in plants. Membrane potential that reduces drastically in plasma membrane of root epidermal cells of rice and saturating nature of Cd uptake indicate the presence of secondary transporter-mediated Cd uptake which are essentially meant for micronutrient uptake (Llamas et al. 2000). Interaction of transporters and chelating agent necessary for metal uptake and storage also supports uptake of Cd through micronutrient transporters (Clemens et al. 2002). Families of transporters that mediate Cd uptake and transport reported include Zip and Nramp (broad range), ABC, Ysl, Copt, Heavy metal ATPases (CPX-type),  $\text{Ca}^{2+}$ -ATPases, Cation/ $\text{H}^{+}$  antiporters, and GNGC (Clemens et al. 2002; Colangelo and Guerinot 2006; Hall and Williams 2003; Williams et al. 2000). Transporter families such as ZIP, COPT, and Nramp were found to be involved in Cd uptake while CDF family and  $\text{P}_1\text{B}$ -ATPases mediate trafficking of Cd including Cd export from cytoplasm and delivery to metal chaperones, respectively (Lee et al. 2007; Shah and Nongkynrih 2007). With rice being graminaceous, targeting of YS1 (OPT) is important in regulating metal uptake because of the chance of competition in Cd uptake with phytosiderophore-mediated iron assimilation (Curie and Briat 2003). Genes found in *T. caeruliensis* such as *TcHMA4* are reported to play a role in xylem loading of Cd (Papayan and Kochian 2004). It is beneficial to manipulate genes involved in xylem loading of Cd that helps to reduce metal uptake to the aerial parts. In a transgenic program which is utilizing metal transporters, the concept of genetically modified rice plants having metal exporters such as bacterial ABC transporters stands among primary targets while overexpression and silencing of transporters of interest to regulate Cd trafficking lies as a secondary choice. Regulation of Cd-specific transporter families at specific tissues will help to control passage of Cd into rice. One disadvantage of this approach is that blockage of broad-range substrate transporters that are involved in Cd uptake causes reduction in uptake of micronutrients too.

Cadmium complexation in plants involves metal binding with chelating agents such as organic acids, small peptides, and metal-binding proteins (Saraswat and Rai 2011; Shah 2011). Most of the chelated toxic metals inside plants are targeting into vacuoles through metal detoxification process (Hall 2002; Haydon and Cobbett 2007). Exudates of root contain metal chelators which play a role in adjustment of rhizosphere pH and metal chelating process (Dong et al. 2007). A study with metal hyperaccumulators points out that chelators secreted by root result in an increase in plant-available Cd whereas toxicity of Cd is alleviated by metal–chelator complex formation (Callahan et al. 2006). Organic acids secreted from roots, e.g., malate, citrate, etc., are involved in metal uptake, long distance transport of metal, and transport of metal into vacuole (Jabeen et al. 2009; Verbruggen et al. 2009). It is found that chelators play a crucial role in keeping Cd in rice roots and form a barrier in Cd translocation (Nocito et al. 2011). Hence, controlled expression of Cd-inducible chelating agents such as phytochelatin, metallothionein, nicotianamine derivatives, etc. and targeting them to a non-edible part of the plant is important for preventing Cd entry into rice grain (Matsuda et al. 2009; Rauser 1999). Chelators are not favored in grains because of the ability to increase metal-holding capacity in grains.

#### 4.2 Cadmium-dependent genomic traits

Difference in metal uptake and allocation observed among rice cultivars enable to develop rice varieties that limit Cd accumulation. Cd uptake and partitioning in rice is found under control of dominant traits which are heritable (Ishikawa et al. 2011; Kato et al. 2010; Ueno et al. 2011). It has been reported that there is a natural variation of about 13–23-fold difference in grain Cd concentration in diverse japonica rice germplasm (Arao and Ae 2003; Ueno et al. 2009). Characteristics found to influence Cd accumulation are high root activity, high shoot-to-root ratio, and water consumption (Hart et al. 1998; Ishikawa et al. 2010). Genetic background of QTLs identified with respect to Cd accumulation enables marker-assisted selection of genes that control low Cd trait in rice grain. Major QTLs controlling Cd accumulation are reported at chromosomes 2, 7, 3, 4, 6, 8, 5, 11, and 10 (Ishikawa et al. 2010; Ueno et al. 2009; Xue et al. 2009). Since the trait was found to be similar in lowland and upland conditions, low Cd trait will persist with regard to different environmental conditions. Utilization of low Cd trait allele will also help to minimize Cd concentration in grain along with little efforts for management practices (Grant et al. 2008). Hence, utilization of QTLs identified as low Cd trait in conjunction with economic traits must be in focus.

Genes identified in response to Cd in plants as well as microbes also open access to genetic recombination in rice for Cd minimization. Transformation of genes such as *MT*, *CUP*,

*gsh*, *APS*, *GR*, *OASTL*, *ArsC*, *GR*, and *ZntA* was found to enhance Cd tolerance, and that of *AtNramp* and *AtPCS* resulted in Cd hypersensitivity in various model experimental plants (Cherian and Oliveira 2005; Ueno et al. 2010). But tolerance phenomenon does not stand for reduction in Cd content in plants and more or less useful for phytoremediation. Transformation and site-specific expression of P-type ATPase genes that code for metallochaperones is promising which helps to traffic Cd to non-edible parts of the rice plant. Transgenics using Cd efflux mechanism that operate through expression of operons controlling *CzcC*, *CzcB*, and *CzcA* proteins in bacteria is another way to prevent entry of Cd into grains (Keller et al. 2005). Recombining with *CadA* or a gene that codes for Cd efflux ATPase and the ABC transporter *AtPDR8*, a Cd extrusion pump are also important to exclude the metal which entered into root (Kim et al. 2007; Silver 1996).

#### 4.3 Cadmium-responsive genes in rice

A number of genes are reported in rice plants that relate with Cd uptake and tolerance (Table 2). Many of these genes are metal transporters which traffic Cd. Apart from metal transporters, genes that are specific to Cd signaling and tolerance are also reported. It is noteworthy that many of the Cd transporters are broad substrate range transporters that are able to transport essential trace elements such as Mn, Fe, and Zn in rice (Ishikawa et al. 2012; Ishimaru et al. 2012; Sasaki et al. 2012; Shimo et al.; 2011). This points to the complexity in modification of metal transporters for reduction in Cd uptake. An approach that pumps out specifically Cd from root is feasible to avoid deficiency of essential nutrients. Exploiting specific transporters that mediate phloem loading of Cd and translocation in phloem is important since grain filling is largely due to phloem loading. One of the drawbacks of ensuring Cd tolerance is that tolerance phenomenon does not account for reduction in Cd uptake. Tolerant varieties usually accumulate more cadmium unless tolerance operates by metal exclusion. Tissue dilution effect needs to be checked in such cases where tolerant varieties dilute amount of Cd accumulated with higher biomass production. A tolerant variety that produces higher biomass and accumulates more amount of Cd in tissues other than grain is preferred.

Cadmium signaling and metallothionein genes are related with maintaining redox balance in cells. These genes are operated as a response to alterations physiological process such as photosynthesis that assures reducing power (Reddy et al. 2004). Hence, the effect of these genes more or less occurs indirectly. For example, expression of MAPK kinases is due to Cd-induced reactive oxygen production and related oxidative stress (Pitzschke and Hirt 2006). Since production of metallothionein depends on the availability of sulfur, genes involved in sulfur metabolism are also indirectly related to Cd-inducible responses (Rauser 1999). Thus, expression of genes under Cd



**Table 2** Genes of rice reported to be regulated by cadmium

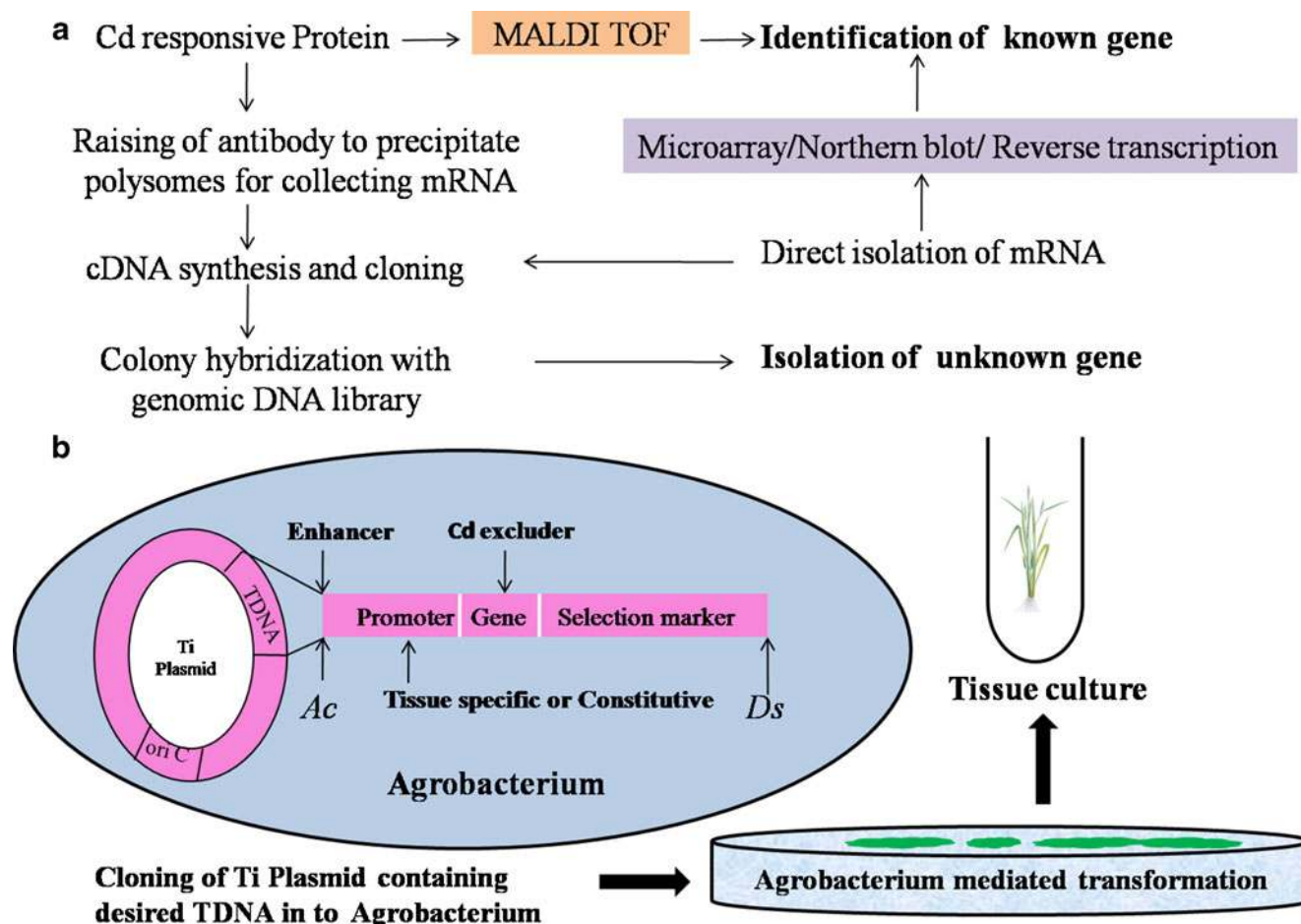
Gene	Function	Reference
<i>OsNramp1</i> , <i>OsNramp 5</i>	Cd, Mn, and Fe transporter	Sasaki et al. 2012; Ishimaru et al. 2012
<i>Os LCD</i>	Cd and Mn transporter	Shimo et al. 2011; Ishikawa et al. 2012
<i>OsLCT1</i>	Cd transporter in phloem	Uraguchi et al. 2011
<i>OsHMA2</i>	Cd and Zn translocation	Takahashi et al. 2012; Satoh-Nagasawa et al. 2012; Nocito et al. 2011
<i>OsHMA3</i>	Sequestration of Cd in root	Takahashi et al. 2012; Ueno et al. 2010; Miyadate et al. 2011
<i>OsCDT1–OsCDT5</i>	Cd uptake inhibitor	Kuramata et al. 2009
<i>OsIRT1</i> , <i>OsIRT2</i>	Cd and iron transporter	Lee and An 2009; Nakanishi et al. 2006
<i>OsNramp1</i>	Cd accumulation in leaf	Uraguchi et al. 2011; Takahashi et al. 2011
<i>OsYSL2</i>	Cd translocation	Masuda et al. 2012
<i>OSISAP1</i>	Cd tolerance	Mukhopadhyay et al. 2004
<i>OsMTP1</i>	Cd translocation	Yuan et al. 2012
<i>Os HsfA4a</i> , ricMT, rgMT	Cd tolerance via metallothionein	Shim et al. 2009; Yu et al. 1998; Hsieh et al. 1995
<i>OsZIP1</i>	Cd and Zn transport	Ramesh et al. 2003; Chou et al. 2011
<i>OsNAAT1</i>	Cd accumulation	Cheng et al. 2007
<i>Ospdr9</i>	Redox protection in Cd stress	Moons 2003
<i>RCS1</i>	Cd complexation via sulfur	Harada et al. 2001
<i>OsMAPK2</i> , <i>OsMSRMK2</i> <i>OsMSRMK3</i> , <i>OsWJUMK1</i>	Cd signaling	Yeh et al. 2004; Agrawal et al. 2002
<i>OsABCG43/PDR5</i>	Cd compartmentalization	Oda et al. 2011
<i>qCdT7</i>	Root–shoot translocation of Cd	Tezuka et al. 2010
<i>PEZ1</i>	Cd accumulation	Ishimaru et al. 2011
<i>OsHMA9</i>	Cd efflux	Lee et al. 2007

stress is in a network that links metabolic pathways in cell and offers identification of a wide variety of genes that are regulated by Cd in the future. This dependency of cellular metabolism on Cd allows metabolic engineering of rice to cope with Cd toxicity. Overproduction of polyamines, phenolics, flavonols, and thiols are some of the metabolic compounds that must be targeted primarily in this aspect since these compounds alleviate Cd stress (Arvind and Prasad 2005).

#### 4.4 Cadmium excluder rice

Since the development of Cd-tolerant plant leads to Cd accumulation rather than Cd exclusion, the concept of excluder plant arises. Generation of Cd excluding rice phenotype through genetic manipulation helps to block entry of Cd into rice plants. A Cd excluder rice plant will be able to survive in the presence of a higher concentration of Cd in soil while accumulating a lower amount of Cd. Naturally occurring Cd excluder phenotypes screened in rice cultivars indicate potential Cd excluding traits in rice genome and offer a vast opportunity for plant breeding to develop Cd excluder rice (Yu et al. 2006; Zhan et al. 2012). One of the disadvantages observed in excluder crops is weak grain production capacity. Thus, identification of Cd exclusion genes and its interaction with yield parameters are important for the development of excluder rice.

If the trait is localized as specific and non-polygenic, incorporation of Cd exclusion genes with increase in yield will be able to solve the issue of decreased yield in excluder rice. Transgenic approach could be either incorporation of genes responsible for Cd exclusion or complexing Cd within root. Techniques such as ion irradiation, activation tagging, marker-assisted breeding, etc. account for promising ways to locate genes that have a specific function such as Cd exclusion (Fig. 5). The first two methods mentioned are useful to develop mutants with desirable trait, i.e., minimization of Cd transport into the grain. Ion irradiation method makes use of energetic heavy-ion beams of charged particles such as carbon, neon, nitrogen, etc. which induce mutations with high frequency at a relatively low dose to plants (Tanaka et al. 2010). Activation tagging method is based on gain-of-function mutagenesis which may be either random insertion of transcriptional enhancers into the genome or expression of genes under the control of a strong promoter with the help of a transposon system (Gou and Li 2012; Lish 2002). Marker-assisted breeding helps to develop rice varieties from the mutants with the help of molecular markers that track the genetic makeup of plants (Collard and Mackill 2008). Thus, molecular techniques offer a vast opportunity to develop Cd excluder rice, but time period stands as a major drawback.



**Fig. 5** Molecular tools and cadmium excluder rice. Cadmium (Cd)-responsive genes can be isolated or identified from expressed protein or mRNA which are regulated during Cd stress in plants (Fig. 3a). Other methods such as map-based cloning, differential display, mutant complementation, subtractive hybridization, etc. are also applicable to isolate Cd-specific genes. Once a desired gene is isolated, transgenic rice plant via classical transformation allows development of Cd excluder rice (Fig. 3b). Method such as activation tagging using enhancer sequence is useful for tagging Cd-responsive genes throughout the plant body or in a specific tissue with usage of a tissue-specific promoter and

hence identify Cd-responsive genes. In this method, addition of a tissue-specific promoter such as *OsGT1* (Wang et al. 2007b) also allows spatial expression of Cd-responsive genes whereas addition of a constitutive promoter such as CaMV5'S allows expression of the gene throughout the plant body. *Ac-Ds* transposon vector system used in activation tagging has the advantage of requiring a minimal number of primary transformants and eliminates the need for crossing. Random mutagenesis methods such as tilling, ion beam irradiation, etc. also open the way to identify Cd-responsive genes as well as development of Cd excluder rice through marker-assisted breeding

#### 4.5 Biofortification

Biofortification enriches micronutrients in cereal grains (Johns and Eyzaguirre 2007; Prasad 2008). Apart from this kind biofortification of rice grain, it may lead to constrained amount of grain Cd too. Biofortified crops having a capacity to specifically uptake and accumulate micronutrients limit entry of Cd into plant tissues (Prasad and Nirupa 2007; Prasad 2008; Zhao and Shewry 2011). Biofortification could be practiced through either usage of micronutrient fertilizers or genetic changes that enhance accumulation of micronutrients. Genetic approach is supported by the finding that transgenic tobacco carrying cDNA *LTC1*, a nonspecific transporter for  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Na}^{+}$ ,

and  $\text{K}^{+}$ , displayed a substantially higher level of tolerance to Cd and accumulated less Cd in roots (Antosiewicz and Henning 2004). It is noteworthy that transgenic approaches to increase micronutrient content of cereal grains often need manipulation of metal transporters. Similarity in both uptake and allocation pattern of Cd with micronutrients such as Zn raises the possibility of technical problems in biofortification such as an increase of Cd content because of manipulation in transporters that are meant for Zn also reflect an increase in Cd uptake (Kramer 2010; Palmgren et al. 2008). On the other hand, boosting of field micronutrients level creates competition in uptake of Cd with micronutrients and hence possibility of reduction in Cd uptake exists.



**Fig. 6** Rice paddies are wetland agroecosystems facing threat of cadmium contamination. This has serious human health implications such as bone mineralization. Excessive use of rock phosphate fertilizer and irrigation with contaminated groundwater must be strictly monitored to ensure food safety. Sustainable agriculture practices (some of which are covered in this paper) minimize Cd translocation in rice fields in an eco-friendly manner

## 5 Outlook

Cadmium minimization must be an important demand during cultivation of rice especially in areas prone to industrial pollution (Fig. 6). Development of biosensors that indicate field Cd level is feasible to screen Cd-contaminated fields on a large scale. Selection of low-cost and widely applicable methods for Cd reduction in rice is preferred. Efficiency of field-based techniques such as application of soil amendments and phytoremediation needs further improvement through technological approach (Prasad 2004; Rajkumar et al. 2012). Establishment of database of amendments will be helpful for immediate remediation programs in contaminated fields. The scope of biofortification of rice with micronutrients that reduce Cd accumulation remains unexplored. Generation of rice mutants and the ability of these mutants in Cd uptake and Cd allocation into grains need to be studied through high-throughput methods (Uraguchi and Fujiwara 2012). Application of knowledge that comes with plant metal transporters must be explored for reducing Cd uptake especially that of silicon which is reported to be essential for the growth of rice (Ma et al. 2006; Vaculik et al. 2012). Transgenic rice having bacterial metal excluder is another promising area to be explored. Vast germplasm available in case of rice plants must be screened for hybridization strategies (Ishikawa et al. 2012). Silencing of Cd uptake genes using repressors is another approach to be studied along with its effect on essential nutrient uptake.

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