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## **Cadmium minimization in wheat: A critical review**

Muhammad Rizwan, Shafaqat Ali, Tahir Abbas, Muhammad Zia-Ur-Rehman,  
Fakhir Hannan, Catherine Keller, Mohammad I. Al-Wabel, Yong Sik Ok

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## Review

### Cadmium minimization in wheat: A critical review



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#### ABSTRACT

Cadmium (Cd) accumulation in wheat (*Triticum aestivum* L.) and its subsequent transfer to food chain is a major environmental issue worldwide. Understanding wheat response to Cd stress and its management for aiming to reduce Cd uptake and accumulation in wheat may help to improve wheat growth and grain quality. This paper reviewed the toxic effects, tolerance mechanisms, and management of Cd stress in wheat. It was concluded that Cd decreased germination, growth, mineral nutrients, photosynthesis and grain yield of wheat and plant response to Cd toxicity varies with cultivars, growth conditions and duration of stress applied. Cadmium caused oxidative stress and genotoxicity in wheat plants. Stimulation of antioxidant defense system, osmoregulation, ion homeostasis and over production of signalling molecules are important adaptive strategies of wheat under Cd stress. Exogenous application of plant growth regulators, inorganic amendments, proper fertilization, silicon, and organic, manures and biochar, amendments are commonly used for the reduction of Cd uptake in wheat. Selection of low Cd-accumulating wheat cultivars, crop rotation, soil type, and exogenous application of microbes are among the other agronomic practices successfully employed in reducing Cd uptake by wheat. These management practices could enhance wheat tolerance to Cd stress and reduce the transfer of Cd to the food chain. However, their long-term sustainability in reducing Cd uptake by wheat needs further assessment.

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## 1. Introduction

Contamination of agricultural soils with heavy metals is a serious threat to crop production worldwide (Adrees et al., 2015a; Ali et al., 2015; Habiba et al., 2015; Rizwan et al., 2016a). Among heavy metals, Cd is highly toxic to plants and animals even at very low concentrations due to its non-essentiality in living organisms. The Cd-contaminated food is the main source of Cd entry to humans via the food chain (Huang et al., 2008; Dai et al., 2012). Cereal crops such as wheat, rice and maize fulfill the major food requirements worldwide. Among cereals, wheat is the staple food for more than half of the world's population with a world production of about 650 million tons per year (FAO, 2012). As compared to other cereals, wheat could accumulate more Cd mainly through roots and could translocate to aerial parts and finally accumulates in wheat grains (Greger and Löfstedt, 2004; Jafarnejadi et al., 2011). Thus, wheat-derived products are the major source of Cd intake by humans. Cadmium is more toxic to wheat as compared to other toxic metals such as chromium (Lopez-Luna et al., 2016). Cadmium toxicity decreased the uptake and translocation of essential elements by plants, including wheat (Yourtchi and Bayat, 2013). Under Cd stress, wheat root growth and morphology is severely affected. In shoots, Cd toxicity showed many physiological disorders such as a decrease in photosynthesis, sugars and soluble proteins and antioxidant enzyme activities (Ci et al., 2009; Shafi et al., 2011; Wang et al., 2011; Li et al., 2015). Cadmium toxicity also increased the production of reactive oxygen species (ROS) which caused oxidative stress in plants (Lu et al., 2013; Chen et al., 2014; Poghosyan et al., 2014; Tauqeer et al., 2016). This would result in a reduction in plant growth, biomass and grain yield (Ci et al., 2010a). However, plant response to Cd toxicity varies among wheat cultivars i.e. durum wheat accumulates higher Cd concentration as compared to bread wheat (Gao et al., 2011a; Jafarnejadi et al., 2011; Erdem et al., 2012; Naeem et al., 2016). Cadmium accumulated in shoots could translocate to grains and then could transfer to humans and animals via the food chain. Thus, Cd minimization in wheat is an important demand especially in soils contaminated with Cd.

Different mitigation strategies have been employed for the reduction of Cd toxicity in wheat. These strategies may include plant growth regulators (PGRs), proper application of mineral nutrients, silicon, inorganic amendments, biochars, manure and compost (Gondor et al., 2014; Asgher et al., 2015; Huang et al., 2015; Ishikawa et al., 2015; Ok et al., 2015; Rizwan et al., 2015). Agronomic management practices such as low Cd-accumulating wheat cultivars, crop rotation, planting patterns and application of microbes might also be used for the reduction of Cd uptake and toxicity in wheat (Ahmad et al., 2015; Greger and Landberg, 2015a; Qiu et al., 2011; Naeem et al., 2016). The objective of the present review is to discuss the Cd toxicity and tolerance mechanisms in wheat and some possible management measures to alleviate Cd toxicity in wheat.

## 2. Cadmium uptake by wheat

Cadmium enters into the soil through geogenic and anthropogenic activities (Boussen et al., 2013). Human activities contribute about 13,000 of the total 30,000 t of annual Cd addition to

the environment (Gallego et al., 2012). Cadmium concentration in agricultural and garden soils ranged from 0.01 to 0.7 and 0.27–2.86 mg kg<sup>-1</sup> respectively (Szolnoki et al., 2013). Cadmium content in phosphate fertilizers ranges between 2 and 200 mg kg<sup>-1</sup> while sedimentary rocks contain Cd about 15 mg kg<sup>-1</sup>. Cadmium availability in soils depends upon a large number of factors such as soil Cd content, soil pH, organic matter, clay minerals, cation exchange capacity (CEC) and type of fertilizers (He et al., 2015; Ran et al., 2016). Jafarnejadi et al. (2011) reported that there was a significant correlation between wheat grain Cd and organic carbon, CEC, and DTPA-extractable Cd concentrations in the soil. Among soil factors, soil pH is the most important factor contributing Cd uptake by wheat plants (Nan et al., 2002; Li et al., 2014; Liu et al., 2015a; Ran et al., 2016).

Cadmium mainly enters in wheat plants through roots (Hart et al., 2006; Adeniji et al., 2010; Black et al., 2014). Root exudation also plays an important role in Cd accumulation by wheat (Cielinski et al., 1998; Greger and Landberg, 2008). Cadmium uptake by wheat varies with soil type, atmospheric pollution and wheat cultivars (Guo et al., 2012; Liu et al., 2015a). More recently, Dahlin et al. (2016) reported that chloride (Cl<sup>-</sup>) could mobilize Cd in the soil and increase its uptake by wheat especially by mobilizing inherent soil Cd. After root uptake, a higher concentration of Cd is accumulated in roots and less is translocated to shoots depending upon wheat cultivars (Adeniji et al., 2010; Ci et al., 2010a). Higher Cd retention in roots might be due to chelation with organic acids as suggested by (Adeniji et al., 2010). However, Hart et al. (2006) reported that phytochelation might not be a limiting factor in the differential storage of Cd in wheat roots.

Cadmium accumulation in wheat shoots depends upon root-to-shoot Cd translocation while accumulation in grains depends upon root-to-shoot Cd transfer and direct pathway of Cd transport from roots to grain via xylem-to-phloem transfer in the stem (Harris and Taylor, 2004, 2013). Quinn et al. (2011) reported that increased shoot Cd content in 'low' isolines of wheat was associated with transpiration rate while in 'high' isolines Cd accumulation was independent of transpiration. Cadmium transport from root to the shoot might be due to symplastic movement of Cd into the root stele as suggested by Van der Vliet et al. (2007). Riesen and Feller, (2005) reported that Cd could be remobilized in wheat through phloem along with transpiration. Chen and Hale (2004) suggested that Cd content in durum wheat grain was a function of total shoot accumulation.

## 3. Toxic effects of Cd in wheat

Cadmium is highly toxic to wheat and reduction in seed germination is the first toxic effect of Cd in wheat. Cadmium, for example, decreased seed germination of four wheat cultivars in a dose and cultivar-dependent manner (Ahmad et al., 2012). Cadmium toxicity to seed germination depends upon the growth media (Ahmad et al., 2013). It has been reported that wheat cultivation on soil had better germination, measured in terms of the plumule and radicle length, as compared to filter paper under same Cd levels which might be due to sorption of Cd on the soil exchange sites (Ahmad et al., 2013).

The major visible symptoms of Cd-induced toxicity in wheat are chlorosis, necrosis, browning of root tips, reduced plant growth

and even plant death especially at higher Cd levels in the growth medium (Ci et al., 2009; Rizwan et al., 2016b). Cadmium toxicity reduced the root and shoot lengths of wheat plants and shoot length was very sensitive to Cd stress as compared to the root length (Cao et al., 2007; Ahmad et al., 2012; Jin et al., 2015). Cadmium toxicity reduced the wheat root growth and affected the root morphology (Wang and Zhou, 2005; Ci et al., 2010a). Excess Cd decreased the leaf number and leaf area of wheat plants (Khan et al., 2007). Excess Cd caused a reduction in shoot and root fresh and dry weights of wheat (Ci et al., 2010a; Riaz et al., 2014; Rizwan et al., 2016b). Cadmium toxicity decreased yield characteristics, ear number, ear weight, ear length, spikelet number and grains per ear, of wheat (Khan et al., 2007). However, the threshold of phytotoxic concentrations of Cd varies with wheat genotypes, the dose and duration of Cd exposure along with other factors.

The presence of Cd caused a reduction in photosynthetic pigments in wheat leaves such as chlorophyll *a*, chlorophyll *b* and carotenoid concentrations (Ci et al., 2010a; Chen et al., 2014). Excess Cd caused a reduction in the leaf net photosynthetic rate (*P<sub>n</sub>*), stomatal conductance (*G<sub>s</sub>*), and transpiration rate (*T<sub>r</sub>*) in a dose and time-dependent manner (Khan et al., 2007; Ci et al., 2010a; Shafi et al., 2011; Li et al., 2015). However, inhibition of gas exchange characteristics differed among wheat genotypes (Shafi et al., 2011).

Cadmium toxicity in the growth medium impaired wheat nutrition and changed nutrient ratios in the wheat tissues (Jalil et al., 1994; Ouzounidou et al., 1997; Rizwan et al., 2016b). Excess Cd decreased the concentration of potassium (K), zinc (Zn) and manganese (Mn) in both roots and shoots of wheat (Jalil et al., 1994). Cadmium toxicity decreased iron (Fe), magnesium (Mg), calcium (Ca) and K concentrations in wheat shoots in a dose-dependent manner (Ouzounidou et al., 1997). Cadmium treatment decreased the macronutrient, N, P, K and Ca, concentrations in wheat plants in a dose-dependent manner (Yourtchi and Bayat, 2013). Cadmium treatment decreased net nitrate accumulation by wheat plants (Stolt and Oscarson, 2002). Cd-mediated reduction in nutrient uptake by wheat might be due to completion of Cd with nutrients during uptake by plants at the root surface and/or by inhibiting the transporters/channels of loading other elements into the aerial part of the wheat. Cadmium toxicity decreased total soluble sugar concentrations while increased the free amino acid concentration in both shoot and root of wheat (Ci et al., 2009).

In conclusion, Cd toxicity decreased wheat growth and biomass by inhibiting the leaf photosynthesis, decreasing gas exchange characteristics and altering the mineral nutrient uptake by wheat. However, wheat response to Cd toxicity varied among genotypes and Cd stress applied.

#### 4. Cadmium toxicity mechanisms in wheat

The Cd-mediated decrease in wheat growth and biomass might be due to different toxicity mechanisms in wheat at physiological and molecular levels. In literature, there is increasing evidence that excessive production of reactive oxygen species (ROS) is the major Cd toxicity mechanism in wheat and other plants (Dey et al., 2007; Chen et al., 2010; Gajewska and Skłodowska, 2010; Wang et al., 2011; Lu et al., 2013). Excess Cd caused overproduction of malondialdehyde (MDA) contents in both shoots and roots of wheat (Dey et al., 2007; Ci et al., 2009; Chen et al., 2010, 2014). Groppa et al. (2008) reported that Cd toxicity increased the Thiobarbituric acid reactive substances (TBARS) contents in wheat plants as compared to control. Khan et al. (2007) reported that the contents of H<sub>2</sub>O<sub>2</sub> and TBARS increased in wheat root and leaf as compared to control but the response varied between two cultivars studies.

Cadmium stress caused membrane structural damage of wheat which might reduce mineral nutrient uptake by plants. For example, Milone et al. (2003) reported that K<sup>+</sup> leakage, a symptom of membrane damage, increased in Adamello cultivar of durum wheat as compared to Ofanto cultivar that indicated free circulation of Cd ions was lower in Ofanto cultivar which might be due to the vacuolar compartmentalization of Cd.

Reduction in photosynthetic pigments might be due to Cd-mediated damage of chloroplasts structure and function of leaf photosystem. It has been reported that Cd stress (50 μM for 24 days) damaged the leaf photosystem II (PSII) (Ci et al., 2009, 2010a). In another study, Cd toxicity damaged the structure of chloroplasts as manifested by the disturbed shape and the dilation of the thylakoid membranes (Ouzounidou et al., 1997).

Cadmium toxic effects on wheat plants might also be due to overproduction of signalling molecules, the subcellular distribution of Cd and structural alterations in proteins. Groppa et al. (2008) suggested that nitric oxide (NO) formation in wheat roots might be involved in root growth inhibition of wheat under Cd stress. Li et al. (2011) reported that Cd concentrations increased in metal sensitive fractions, heat-denatured protein+organelles while decreased in detoxified fractions, heat-stable protein+Cd-rich granule, with increasing Cd levels in the growth medium. The authors suggested that Cd toxicity to wheat roots strongly depends upon subcellular Cd distribution. Excess Cd caused structural alterations in proteins and inhibited the activities of certain genes (Cebeci et al., 2008).

In a word, overproduction of ROS, structural alterations and genotoxicity are the main mechanisms causing Cd toxicity in wheat plants.

#### 5. Cadmium tolerance mechanisms in wheat

Wheat plant has evolved different tolerance mechanisms to avoid harmful effects of Cd stress in plants (Ge et al., 2009; Chen et al., 2010). Ci et al. (2009) reported that Cd uptake and translocation was lower in wheat Cd-tolerant lines as compared to Cd-sensitive lines. Similar results were observed in other studies (Zhang et al., 2000, 2002; Khan et al., 2007). Cadmium concentration was significantly higher in roots than the aerial parts of wheat (Liu et al., 2009). Ci et al. (2011b) reported that Cd root-to-shoot translocation was lower in Cd-tolerant varieties as compared to Cd sensitive varieties and wheat varieties in different decades were different in Cd tolerance and accumulation. These studies showed that lower Cd uptake and accumulation might be a tolerance mechanism in wheat cultivars.

Enhancement in antioxidant enzyme activities is one of the main Cd tolerance mechanisms in plants including wheat (Mühling and Läuchli, 2003; Khan et al., 2007; Chen et al., 2010; Poghosyan et al., 2014). There are two types of antioxidant enzymes including enzymatic and non-enzymatic antioxidants to quench ROS in wheat under Cd stress. The activities of superoxide dismutase (SOD), catalases (CAT), and peroxidases (POD) increased in wheat shoots and roots with increasing Cd concentrations in the soil up to 10 mg Cd kg<sup>-1</sup> of soil and then decreased with higher Cd levels in the growth medium (Chen et al., 2014). Under 50 μM Cd stress for 24 days, leaf CAT activity decreased in the Cd-sensitive lines of wheat while increased in tolerant lines (Ci et al., 2009). Lower Cd concentrations (less than 3.3 mg kg<sup>-1</sup>) did not change the activities of SOD, CAT, guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR) but increased at 10 mg Cd kg<sup>-1</sup> of soil and then decreased with higher Cd levels (Lin et al., 2007). When compared with control, APX activity increased in wheat leaves at moderate Cd level (2.69 mg kg<sup>-1</sup>) while decreased at a higher Cd stress (9.48 mg kg<sup>-1</sup>) level in the soil (Li



et al., 2014). Milone et al. (2003) reported that APX, GPX and syringaldazine peroxidase (SPOD) activities were always higher in roots than in leaves under Cd stress. These enzymes activities increased in leaves in Ofanto cultivar under Cd stress while in Adamello cultivar leaves they remained at control levels or slightly increased especially at highest Cd levels. Yannarelli et al. (2007) reported that glutathione reductase (GR) activity increased in wheat roots under Cd stress. Above studies indicated that Cd tolerance in wheat might be related to the activation of the anti-oxidant system but the response varied among wheat genotypes.

Over production of phytochelatin (PC) also play a vital role in Cd tolerance in wheat (Keltjens and Beusichem, 1998; Stolt et al., 2003; Ranieri et al., 2005; Ahmad et al., 2009). Excess Cd increased the synthesis of glutathione (GSH), a low molecular mass compound, in wheat plants (Lin et al., 2007; Groppa et al., 2008; Ahmad et al., 2009). Cd treatment stimulated the activity of glutathione S-transferase (GST) in wheat (Wang et al., 2011). In another study, Cd stress increased the production of non-protein thiols (NPT) and GSH contents and GST activity in shoots and roots of wheat (Gajewska and Skłodowska, 2010). It has been reported that phytochelatin (PC-SH) concentration increased in both shoots and roots of wheat plants up to 2.0  $\mu\text{M}$  Cd in solution while PC-SH concentrations remained constant or slightly decreased at higher Cd levels in the solution (Keltjens and Beusichem, 1998). Under Cd stress, metabolites of the ascorbate (ASC)–glutathione (GSH) cycle increased in wheat roots as compared to control (Paradiso et al., 2008).

The plant could also tolerate excess Cd by modulating plant growth regulators (PGR) in plants through different mechanisms. In plants, major PGRs include salicylic acid (SA), gibberellic acid (GA), ascorbic acid (AsA), abscisic acid (ABA), jasmonic acid (JA), Indole-3-acetic acid (IAA), cytokinins (CKs), ethylene, nitric oxide (NO), brassinosteroids (BRs) and polyamines (PAs) (Asgher et al., 2015). Studies reported that wheat could tolerate excess Cd by enhancing the production of PGRs under Cd stress. For example, AsA concentration increased in wheat plants under Cd exposure as compared to control (Riaz et al., 2014). In addition, SA synthesis increased in wheat roots and especially in leaves under Cd stress as compared to control (Kovacs et al., 2014). Proline accumulation increased in wheat seedlings under Cd stress (Tripathi et al., 2013).

Excess Cd-induced or up-regulated more proteins in wheat leaves which could play important roles in the biochemical reactions involved in Cd tolerance in wheat under Cd stress (Ge et al., 2009). In addition, Wang et al. (2011) reported that Cd stress up-regulated proteins in wheat plants mainly involved in Cd detoxification and antioxidant processes. Ci et al. (2012) identified the quantitative trait loci (QTL) for Cd tolerance and accumulation in wheat using 103 recombinant inbred lines (RILs) derived from a cross of Ch  $\times$  Sh at germination and seedling stages. Results showed that 26 QTLs were detected of which 16 were designed for traits under control and Cd stress, 8 for Cd tolerance and 2 for root Cd accumulation. QTLs detected for the traits were different under control and Cd stress at both germination and seedling stages.

To this end, Cd tolerance mechanisms in wheat plants mainly include exclusion at the root level, compartmentalization in the plants, enhancement the activities of antioxidant enzymes, over production of PGRs, ion homeostasis, production of stress proteins and activation of certain genes. However, these mechanisms vary with cultivars, growth conditions, growth stage and the duration of Cd stress applied. In addition, these mechanisms work for a certain Cd levels and fail to reduce Cd toxicity under higher Cd stress.

## 6. Management of Cd stresses in wheat

As reported above, wheat can accumulate Cd in grains and Cd

could transfer to humans via the food chain. Thus, reduction of Cd in wheat is among the major issues for sustainable agriculture and human health. During the past decades, several practices have been employed in the management of Cd toxicity in wheat. In the following sub-sections, we comprehensively reported the management of Cd in wheat in the light of the published reports.

### 6.1. Exogenous application of plant growth regulators

Plants have evolved tolerance mechanisms to cope with Cd stress, via the expression and regulation of PGRs (Asgher et al., 2015). As reported above wheat plant has developed internal defense mechanisms to cope with Cd stress via the production of phytohormones. However, the efficacy of the endogenous defense system decreased with higher Cd stress. Thus, exogenous application of phytohormones may enhance wheat tolerance to Cd stress. In a study carried out by Shakirova, 50- $\mu\text{M}$  SA pretreatment decreased Cd accumulation in Cd-stressed (1 mM) wheat shoots and decline the level of MDA and electrolyte leakage. Furthermore, SA pretreatment decreased the Cd-induced accumulation of ABA and reduced the fall of IAA and CK in Cd-stressed plants (Shakirova et al., 2016). In Cd-stressed wheat seedlings, ABA and GA application decreased soluble protein contents and increased free proline and soluble phenolics as compared to control (Ergun and Oncel, 2012). Pretreatment with SA (500  $\mu\text{M}$  for 20 h) increased relative water content, chlorophyll contents,  $^{14}\text{CO}_2$  fixation, activities of phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase and abscisic acid while decreased MDA,  $\text{H}_2\text{O}_2$  and free proline contents in Cd-stressed wheat seedlings (Moussa and El-Gamal, 2010a, 2010b). SA pretreatment ameliorated Cd-induced obvious ultrastructural changes in wheat seedlings (Moussa and El-Gamal, 2010b). In another study, the combined application of NO and SA increased plant growth, biomass, chlorophyll contents, mineral nutrients and enhanced the activities of SOD, POD and CAT in Cd-stressed wheat seedlings (Basalah et al., 2013). Pretreatment with IAA or SA mitigated the Cd-induced reduction in wheat growth, photosynthesis, activities of antioxidant enzymes and leaf structure (Agami and Mohamed, 2013).

In Cd-stressed wheat seedlings, NO, as sodium nitroprusside, application decreased oxidative stress and the activities of antioxidant enzymes in roots (Singh et al., 2008). Allagulova et al. (2015) reported that pretreatment with 0.4  $\mu\text{M}$  24-epibrassinolide (EB) increased the accumulation of 28 kDa dehydrin protein in wheat seedlings. Pretreatment with 28 nM wheat germ agglutinin decreased Cd concentrations in roots and shoots and increased plant growth and biomass (Bezrukova et al., 2011). Presoaking wheat seeds with polyamines, spermidine or spermine, increased the seedling growth, membrane stability index, relative water content, concentrations of protein, starch, ascorbic acid and total glutathione while decreased electrolyte leakage, concentrations of proline, total soluble sugars, MDA,  $\text{H}_2\text{O}_2$  and  $\text{Cd}^{2+}$  under Cd stress (Rady and Hemida, 2015). Similarly, GB application increased wheat growth and decreased the oxidative stress, but the response varied between cultivars (Rasheed et al., 2014).

In conclusion, pretreatment with PGRs decreased Cd concentrations while increasing growth, biomass, photosynthesis and maintained the nutrient homeostasis in Cd-stressed wheat seedlings. Exogenous application of PGRs inhibited the oxidative burst in wheat cells and enhanced the activities of antioxidant enzymes of Cd-stressed wheat plants. However, further studies are needed to evaluate the role of PGRs in translocation of Cd from roots to shoots and from shoots to grains.

## 6.2. Use of inorganic amendments

Several inorganic amendments have been employed for the reduction of Cd uptake and toxicity in wheat. For example, Huang et al. (2015) reported that H<sub>2</sub>S pretreatment increased wheat seed germination under Cd stress by modulating antioxidant defense system. In addition, pretreatment with the SO<sub>2</sub> donor, Na<sub>2</sub>SO<sub>3</sub>/NaHSO<sub>3</sub> (3:1), increased wheat seed germination under Cd stress in a dose-dependent manner, optimum concentrations between 1 and 2 mM, by increasing the activities of antioxidant enzymes in germinating seeds (Hu et al., 2015). Rehman et al. (2015) reported that elemental S application in Cd-contaminated soil increased Cd concentration in wheat grains under rice-wheat cropping systems while gypsum application decreased Cd in wheat grains and increased plant growth and biomass. These studies showed that S forms, H<sub>2</sub>S, SO<sub>2</sub> and elemental S, application timing and growth conditions play an important role in reducing Cd stress in wheat. Tlustos et al. (2006) reported that liming the Cd-contaminated soil, 3 g CaO and 5.36 g CaCO<sub>3</sub> per kg of soil, increased soil pH up to 7.3 compared to 5.7 in the control treatment. Liming decreased mobile Cd in the soil and decreased Cd contents in straw and wheat grains. This showed that liming might be another option to reduce Cd uptake by wheat, but its feasibility to the soil as an amendment for metal reduction depends upon the soil buffering capacity.

### 6.2.1. Mineral fertilization

Proper application of different fertilizers might be an effective strategy in reducing Cd uptake and accumulation in wheat by changing Cd fractions in soil and bioavailability to crops. The role of some important mineral elements in reducing Cd accumulation in wheat is described below.

**6.2.1.1. Nitrogen.** Nitrogen, a macronutrient for plants, the application may affect grain nutritional and bread-making quality (Gao et al., 2012). Several studies reported that Cd availability in soil and accumulation by wheat is greatly influenced by N application in the soil (Gray et al., 2002; Li et al., 2013; Ishikawa et al., 2015). Gao et al. (2010) conducted a three-year field trial to determine the effect of source, timing and placement of N fertilizer on Cd concentration in durum wheat grains under reduced and conventional tillage practices. Results showed that N application increased grain Cd concentrations and decreased Zn concentrations in grains and there was a significant year-to-year variation in grain Cd accumulations. Ammonia fertilization increased grain Cd concentrations as compared to urea ammonium nitrate, urea and ammonium nitrate. A banded application of fertilizers increased Cd concentration as compared to dual-banded placement which indicated higher N availability in the banded application. Cd concentrations were higher in grains grown on sandy loam soil as compared to clay loam and reduced tillage decreased grain Cd concentrations as compared to conventional tillage practices. Furthermore, NH<sub>4</sub>Cl fertilizer, basal dressing, increased Cd concentration in wheat grains as compared with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> or urea fertilizers in both field and pot experiments. However, NH<sub>4</sub>Cl application at tillering-jointing and flowering stages had nearly equal effects on Cd concentrations in grains as compared to other fertilizers (Ishikawa et al., 2015). The authors suggested that increase in Cd concentration with NH<sub>4</sub>Cl application might be due to the Cl<sup>-</sup> presence in the soil, which increased Cd mobility and plant uptake as compared to other fertilizers.

In addition, that Cd concentration increased in wheat grains with increasing N rate, irrespective of both Cd concentration in soil and grains (Wangstrand et al., 2007; Li et al., 2011). The response of N towards Cd accumulation in wheat grains varied between cultivars, seeding date, year-to-year wheat growth, and across the

sites (Wangstrand et al., 2007; Perilli et al., 2010). Li et al. (2013) reported that single N application (120 kg ha<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>) at the seeding stage decreased Cd concentration in wheat grains as compared to equally split N applications (NH<sub>4</sub>NO<sub>3</sub> plus calcium ammonium nitrate as a side dressing) in field trials. Gao et al., (2011b) reported that either Cd or Zn positively correlated with grain N concentration among 13 wheat cultivars. Increase in Cd concentration with ammonium fertilizers might be due to decrease in soil pH, nitrification and/or NH<sub>4</sub> uptake by plants as compared to other N fertilizers. Increased Cd concentrations in wheat grains might also be due to increase in Cd concentrations in soil solution through ion exchange reactions. Overall, above studies showed that N fertilizer type, soil type, tillage practices, and application methods affect Cd concentrations in wheat grains. Thus, selection of a suitable N source, application timing and placement is very important in minimizing wheat grain Cd concentrations.

**6.2.1.2. Zinc.** Zinc, a micronutrient for plants, has physical and chemical properties almost similar to Cd and can compete for the binding sites both in the soil and root surfaces for plant uptake. Several studies reported that Zn application decreased Cd concentration in wheat (Hart et al., 2005; Sun et al., 2005b; Liu et al., 2007; Zhao et al., 2011; Erem et al., 2012; Li and Zhou, 2012; Singh and Shivay, 2013). Liu et al. (2007) reported that Zn application decreased Cd concentration in durum wheat seedlings as compared to only Cd-stressed wheat. Saifullah et al. (2014) suggested that foliar application of 0.3% Zn in the form of ZnSO<sub>4</sub> effectively ameliorated the adverse effects of Cd and decreased grain Cd concentration in wheat grown in Cd-contaminated soil. In addition, foliar applied Zn (6.0 g L<sup>-1</sup> as ZnSO<sub>4</sub>) at booting stage increased nutrients (P, K and Na), gas exchange characteristic (photosynthetic rate, transpiration rate and stomatal conductance), and chlorophyll contents in Cd-stressed wheat seedlings (Sarwar et al., 2015). Hart et al. (2002) reported a competitive interaction between Cd<sup>2+</sup> and Zn<sup>2+</sup> at the root cell plasma membrane of both bread and durum wheat. In addition, Zn concentration (≥ 1.0 μM) decreased the Cd translocation from one root section to the other and thus inhibiting the movement of Cd via phloem in durum wheat (Welch et al., 1999). Similarly, Zn application decreased Cd translocation from roots to shoots and decreased oxidative stress as measured by H<sub>2</sub>O<sub>2</sub> and MDA contents (Zhao et al. 2005a, 2005b). Green et al. (2003) reported that Cd translocation to wheat shoots decreased with increasing Zn<sup>2+</sup> activity in the nutrient solution. Koleli et al. (2004) suggested that Zn-mediated alleviation of Cd toxicity in durum wheat might be due to improving in antioxidative defense and/or Zn competing with Cd for binding to critical cell constituents in Cd-stressed wheat seedlings.

The lower level of Zn increased the activities of CAT and APX in Cd-stressed wheat roots while higher Zn decreased enzyme activities, but this increase was dependent on the crop genotype and Zn levels in the media (Sanaeiostovar et al., 2012). Decrease in Cd concentrations in durum wheat grains might be due to competitive inhibition of Cd loading into phloem sap by Zn as suggested by Cakmak et al., (2000a). Harris and Taylor (2001) reported that the simultaneous application of Cd and Zn to the flag leaf inhibited the Cd translocation to grains. Salah and Barrington (2006) reported that application of 5.6 mg kg<sup>-1</sup> of soil decreased Cd uptake by wheat plants while higher Zn concentrations increased Cd uptake by wheat plants causing a synergistic effect in Cd uptake.

In conclusion, Zn supply might be effective in reducing Cd concentrations in wheat seedlings and translocation to grains. However, the Zn response towards Cd toxicity varies with genotypes, the dose and duration of the Zn and Cd exposure. Furthermore, the higher Zn concentration has been itself toxic to plants, thus, it is rarely possible to reduce Cd stress in wheat plants only

by the application of Zn in Cd-contaminated soils. More studies are required to investigate the proper Zn/Cd ratios required to reduce Cd toxicity in wheat.

**6.2.1.3. Phosphorus.** Reduction of Cd toxicity in wheat seedlings by using P containing amendments is well documented. For example, it has been reported that mono ammonium phosphate (MAP) application in soil decreased Cd concentration in wheat grains and increased plant growth, biomass and gas exchange characteristics (Arshad et al., 2016; Rehman et al., 2015). Contrarily, P fertilizer application in the Cd-contaminated field increased the Cd concentrations in durum wheat grains in a dose and site-dependent manner (Grant et al., 2013). In addition, P application increased Cd concentrations in wheat shoots but decreased in roots (Zhao et al., 2005b). Shi et al. (2015) reported that Cd concentrations in wheat grains correlated with P concentrations in wheat straw and grain.

Jafarnejadi et al. (2011) reported that overusing of P-fertilizers increased the Cd concentrations in the topsoil and wheat grains in the study area. This showed that P fertilizers must be used with caution in Cd-contaminated soils in order to reduce Cd entry to food chain through crops. Wang et al. (2014) conducted a long-term (over 20 years) field trial with a crop rotation of winter wheat and summer maize with seven fertilizer treatments in different combinations. Results showed that the Cd level increased in soil with long-term P fertilization as compared to other treatments but Cd uptake by crops decreased which might be due to the precipitation of Cd with phosphate in the soil.

**6.2.1.4. Selenium.** Khan et al. (2015) reported that Se or S application alleviated Cd-induced oxidative stress in wheat seedlings by increasing proline accumulation. Se and S application decreased ethylene level and increased the activity of GR and GPX in Cd-stressed wheat seedlings. The authors suggested that Se and S decreased Cd-induced oxidative stress in wheat by regulating ethylene production, proline, GSH metabolism, and photosynthetic processes. In addition, Zembala et al. (2010) have shown that Se improved nutrients, reduced the lipid peroxidation, Cd concentrations, and exerted positive effects on cell membrane stability of Cd-stressed wheat seedlings. However, the response varied with genotypes i.e. Polish genotype was more tolerant to Cd stress than Finnish one. Contrarily, Se application, regardless of Se form and rate of application, did not reduce the Cd concentration in the durum wheat grain grown under Cd-contaminated field (Grant et al., 2007).

To this end, P amendments must be used with caution due to the presence of Cd in phosphate rocks and long-term P fertilizers should be avoided in already Cd-contaminated soils. Se may protect wheat seedlings against Cd-induced oxidative stress and a decrease in plant growth and biomass but the response varied with growth conditions and genotypes. Thus, more studies are still needed to better understand Se-mediated alleviation of Cd toxicity in wheat plants.

**6.2.1.5. Other minerals.** Bao et al. (2012) reported that Cd accumulation and phytosiderophores (PS) release increased in Fe-deficient wheat seedlings as compared to Fe-sufficient plants. It has been reported that Mg deficiency enhanced the wheat tolerance to Cd toxicity which could partially be ascribed to the preservation of Fe nutrition (Nikolic et al., 2014). Thus, proper Fe and other mineral nutrition may enhance wheat tolerance to Cd stress.

## 6.2.2. Silicon application

Silicon, a beneficial element for crop plants, has been shown to alleviate the abiotic stresses in many plant species, including heavy metal stress (Adrees et al., 2015b; Keller et al., 2015; Greger et al., 2016; Rizwan et al., 2016b). Silicon application increased

growth, biomass, photosynthetic pigments of both bread and durum wheat cultivars grown under Cd stress. Silicon application decreased Cd uptake and translocation in wheat seedlings under Cd stress (Hussain et al., 2015; Naeem et al., 2015; Rizwan et al., 2015). Cadmium concentrations decreased in wheat grains with Si application as compared to untreated control (Hussain et al., 2015; Naeem et al., 2015). Silicon application decreased MDA and H<sub>2</sub>O<sub>2</sub> contents and increased SOD, POD and CAT activities in both leaves and roots (Hussain et al., 2015). The Si-mediated decrease in Cd concentrations in wheat seedling might be due to increase in soil pH (Rizwan et al., 2012), and/or decrease in extractable Cd concentrations in the soil and by increasing Cd accumulation in roots (Rizwan et al., 2012; Naeem et al., 2015). It has been reported that field application of different Si sources increased Si concentrations in wheat while decreased Cd concentrations in edible parts but did not effect the soil bioavailable Cd in the soil (Greger and Landberg, 2015b). Overall, Si response to Cd-stressed wheat varies with cultivars, growth condition, dose and duration of Cd exposure. Thus, further studies are required for better understanding the mechanisms behind Si-mediated alleviation of Cd toxicity in wheat seedling, especially at molecular and proteomic levels.

## 6.3. Use of organic amendments

### 6.3.1. Composts and manures

Organic manures and composts are known to improve nutritional status and structure of soils and as a result, plant growth and biomass are increased due to the availability of nutrients. On the other hand, manures and composts are being used for the reduced heavy metal uptake by plants by immobilizing metals in the soil. Ahmad et al. (2015) reported that compost and biogas slurry application increased wheat growth and biomass and decreased Cd uptake by plants under Cd stress. The response of compost was better in reducing Cd uptake by wheat as compared to biogas slurry but it increased Cd translocation to shoots. Similarly, farm yard manure application led to lower Cd concentration in wheat grains as compared to the control but the response varied with types of the soil (Dahlin et al., 2016). It has been reported that exogenous application of organic chelators decreased Cd toxicity in the wheat shoot and root either by affecting Cd uptake and distribution in plants (Li and Zhou, 2012) or by affecting the PCs synthesis in plants (Sun et al., 2005a). Yousaf et al. (2016) reported that farm manure and sewage sludge application increased Cd uptake by wheat plants as compared to the control and other amendments applied. The increase in Cd uptake by wheat with amendments might be due to the higher Cd contents present in the amendments. Thus, organic amendments used for the immobilization and uptake of Cd by plants must be used after careful analysis of the amendments to avoid the risk of secondary Cd pollution in the soil.

Overall, compost and manure application in the soil decreased and/or increased Cd concentrations in wheat grains depending upon the compost and manure types, application durations and soil types etc. However, studies related to long-term stabilization of Cd by the application of different organic amendments are still needed.

### 6.3.2. Biochar

Biochar, organic material pyrolyzed under limited oxygen supply, the application could decrease Cd and other metal toxicity in many plant species including wheat (Mahmoud et al., 2011; Khaliq et al., 2015; Khan et al., 2015; Ok et al., 2015; Rizwan et al., 2016c, 2016d). Several previous studies have shown that biochar application increased wheat biomass and yield either under normal or Cd-stressed conditions (Cui et al., 2012; Zheng et al., 2013). When used as soil amendment, biochar (prepared from rice straw,



husk and bran at 500 °C for 4 h) application in historically contaminated clay loam soil increased wheat growth and biomass while decreased Cd concentration in shoots by 71% as compared to control. Biochar application decreased  $\text{NH}_4\text{NO}_3$ -extractable Cd and other metals in the soil especially with straw biochar (Zheng et al., 2013). Yousaf et al. (2016) reported that the maximum decrease in the AB-DTPA extractable Cd was due to BC application (*Eucalyptus saligna* wood, 450 °C) as compared to the compost while farm manure and press mud increased available Cd. In addition, Mahmoud et al. (2011) reported that rice husk biochar (450 °C for 15 min) increased wheat biomass and grain yield and greatly decreased the Cd concentrations in both straw and grains as compared to rice husk and control. Cui et al. (2012) studied the effect of biochar application on wheat growth and yield grown in a Cd-contaminated field. Results showed that biochar amendment increased soil pH and decreased  $\text{CaCl}_2$ -extractable Cd concentrations in soil. The biochar increased plant biomass and grain yield while decreased Cd concentrations in plants as compared to control.

Decrease in Cd uptake by wheat plants might be due to the higher surface area, total pore volume and adsorption of Cd with biochars (Xu et al., 2014). The biochar-mediated decrease in Cd concentration in wheat might be due to high pH, porosity, CEC and active functional groups of biochar along with improvement in soil structure and cycling of some mineral nutrients. However, biochar potential towards Cd immobilization and plant uptake varies with feedstock types, processing and growth conditions (Mahmoud et al., 2011; Sigua et al., 2015; Xu et al., 2014). Thus, biochar application with other amendments such as compost and/or mineral fertilization might be a useful option for the remediation of Cd-contaminated soils.

#### 6.4. Selection of low Cd-accumulating wheat cultivars

Selection and breeding of low Cd-accumulating wheat cultivars might be useful for the reduction of Cd accumulation in wheat (Ci et al., 2010b, 2011a; Zimmerl et al., 2014; Liu et al., 2011, 2014; Shi et al., 2015; Kubo et al., 2016). It is well documented that Cd accumulation in wheat straw and grains varied greatly among wheat cultivars even under the same environmental conditions (Bermudez et al., 2011; Kubo et al., 2011, 2016; Arduini et al., 2014; Qiu et al., 2011; Perrier et al., 2016). Cakmak et al., (2000b) reported that there are genotypic differences in Cd binding to wheat cell walls and Cd transport to sink might be affected by the composition of phloem sap ligands. Studies reported that Cd accumulation was higher in grains of durum wheat compared with bread wheat and differences were related to variations in the translocation from root to shoot and within the shoot (Greger and Löfstedt, 2004; Harris and Taylor, 2004; Ganeshan et al., 2012).

Ci et al. (2010b) reported that Cd uptake and accumulation in roots and shoots and translocation to shoots decreased, in general, from octoploid to diploid varieties grown under controlled conditions. Thus, common wheat species, hexaploid, could accumulate more Cd in grains. Arduini et al. (2014) reported that the grain Cd concentration was higher in Svevo than in Creso durum wheat cultivar grown on Cd-spiked soil. The authors suggested that reduced plant height, high root to shoot biomass ratio during vegetative growth promoted Cd accumulation into the grain. It has been reported that low grain Cd concentration in durum wheat might be controlled by a single dominant gene (*Cdu1*) and the adaptation of *th* gene might be responsible for lower Cd uptake by wheat (Knox et al., 2009). Stolt et al. (2006) reported that the genetic variations in the wheat shoot and grain Cd accumulation remained consistent, regardless of soil type or growing season.

Above studies suggested that some potentially useful low Cd-accumulating wheat cultivars could be used in wheat production

on Cd-contaminated soils. The breeding approach might be an option to reduce Cd concentration in wheat grains and screening of low Cd-accumulating wheat varieties, which could ensure food safety. However, little is known about practical techniques in breeding the development of low Cd wheat cultivars.

#### 6.5. Agricultural practices

Agricultural practices may reduce Cd concentration in wheat seedlings (Kubo et al., 2008; Gao and Grant, 2012; Tang et al., 2012). Kubo et al. (2008) reported that Cd concentrations varied in wheat grains with growing seasons and experimental fields. Tang et al. (2012) reviewed that co-cropping with phytoextraction plants and food crops may reduce Cd concentrations in food crops. Wu et al. (2003) reported that wheat/rice intercropping reduced the Cd concentrations in wheat and rice shoots and reduced the Cd concentrations in wheat grains as compared to monoculture plants. Thus, co-cropping of wheat with Cd-hyperaccumulating plants might be an option to reduce Cd concentrations in wheat grains. Similarly, adjusting soil pH may also decrease Cd concentrations in wheat by immobilizing Cd in the soil (Tlustos et al., 2006).

Pre-cultivation with Cd-hyperaccumulating plants may reduce Cd concentration in post-cultivated wheat (Ibaraki et al., 2014; Greger and Landberg, 2015a). It has been reported that cultivation of indica rice varieties, CHOKOUKOKU and IR8, decreased Cd concentrations in wheat varieties, CHUGOKU165 and SHIROGANekomugi, grown after rice cultivation. Between wheat varieties, CHUGOKU165 performed better than the other cultivar. The authors suggested that the terminal point of phytoextraction is when the soil Cd concentration is less than  $0.6 \text{ mg kg}^{-1}$  (Ibaraki et al., 2014). In another study, Greger and Landberg (2008) showed that pre-cultivation of *Salix* significantly decreased the Cd concentration in post-grown wheat grains under field conditions. A high density of *Salix* plantation decreased Cd in wheat grains faster than a low-density plantation.

These studies showed that pre-cultivation with phytoextraction might be effective in reducing Cd concentrations in wheat grains. However, more studies are required to identify other plant species for phytoextraction of Cd before wheat cultivation grown in different soils varying in Cd contents and diverse environmental conditions.

Tillage practices may also be effective in reducing Cd concentrations in wheat grains. For example, it has been reported that the Cd concentration in wheat grains reduced in reduced tillage as compared to conventional tillage (Gao and Grant, 2012). Leaf applied sodium chloride enhanced Cd concentrations in wheat grains (Ozkutlu et al., 2007). This indicated that application of saline waters containing high chloride may mobilize Cd in the soil and increased its uptake by plants.

Selection of proper soil type may reduce Cd concentration in wheat grains. For example, Cieslinski et al. (1996) reported that wheat cultivars were grown in Chernozemic soil accumulated more Cd in grains than plants are grown in the Orthic Gray Luvisol soil. Rojas-Cifuentes et al. (2012) suggested that matching durum wheat with the proper soil series might be the best management practice to produce seed with low Cd concentrations.

#### 6.6. Exogenous application of microbes

Exogenous application of microbes can enhance wheat tolerance to Cd stress. For example, Ahmad et al. (2015) reported that Cd-tolerant bacteria, isolated from rhizosphere soil receiving tannery effluent, increased plant growth, biomass, and relative water content and decreased oxidative stress in Cd-stressed wheat seedlings. However, the bacterial response to Cd stress varies

among bacterial strains in promoting wheat growth and lowering Cd uptake. In another study, root endophyte and arbuscular mycorrhizal fungi application increased Cd-stressed wheat growth, chlorophyll contents, and increased Cd in roots while decreased in shoots especially under higher Cd concentrations (Shahabivand et al., 2012). More recently, it has been reported that the application of plant growth promoting rhizobacteria increased the wheat growth, photosynthesis and decreased Cd uptake both in shoots and roots (Hassan et al., 2016). These studies showed that microbes could be used for the reduction of Cd toxicity in wheat plants. Rhizobia along with other amendments such as biochar enhanced the growth and yield of crops under either normal or stressful conditions (Ahmad et al., 2015; Akhtar et al., 2015). Further studies are needed to reduce Cd toxicity in wheat by combining application of microbes and other amendments such as biochar.

### 6.7. Microwave and laser seed pretreatment

Microwave and laser pretreatment increased wheat tolerance to Cd stress. It has been reported that He–Ne laser pretreatment of wheat seeds decreased MDA, H<sub>2</sub>O<sub>2</sub> contents and production rate of O<sub>2</sub><sup>-</sup> and increased the gene expression levels of SOD, POD, APX, the activities of SOD, POD, CAT, APX, AsA and GSH concentrations as compared to control (Qiu et al., 2013a). Qiu et al. (2011) reported that wheat seeds treated with microwave (5 or 10 s with wavelength 125 mm, power density 126 mW cm<sup>-2</sup>, 2450 MHz) increased plant height, root length, and dry weights. Seed pretreatment decreased MDA, H<sub>2</sub>O<sub>2</sub> contents and production rate of O<sub>2</sub><sup>-</sup> and increased the activities of SOD, POD, CAT, APX, AsA and GSH concentrations as compared to control. In another study, Qiu et al. (2013b) suggested that microwave pretreatment alleviated the Cd-induced toxicological effects on wheat seedlings by the way of a pathway involving nitric oxide. These studies showed that seed pretreatment might be an option for decreasing Cd toxicity in wheat seedlings.

## 7. Conclusion and perspectives

Cadmium is one of the major inorganic contaminants in the environment. Its presence in the soil or atmosphere has been recognized as a serious threat to agriculture. Cadmium toxicity reduced growth and yield of wheat. Excess Cd decreased the photosynthesis, gas exchange parameters and mineral nutrient uptake by wheat. Cadmium toxicity caused oxidative stress and genotoxicity in wheat. However, the severity of Cd toxicity depends on Cd contents in the growth medium, duration of exposure, and plant growth stage. Wheat counteracted the Cd toxicity by stimulation of antioxidant defense system, osmoregulation, ion homeostasis and over production of signalling molecules. Several Cd strategies have been employed for successful mitigation of Cd uptake and toxicity in wheat. These mitigation strategies mainly include the exogenous application of PGRs, proper application of essential nutrients, inorganic amendments such as liming materials and silicon, and organic amendments such as compost, manure and biochar. Selection of low Cd-accumulating cultivars, co-cropping, soil type, exogenous application of microbes and seed pretreatment with microwave and laser have been employed for the alleviation of Cd toxicity in wheat. Cultivation of low Cd-accumulating wheat cultivars along with in situ stabilization and proper management might be effective strategy aiming to obtain grain with low Cd concentrations.

Although several management options have been reported in the literature but further multiple benefits could be achieved by future critical research efforts including the following.

- Cadmium toxicity pathways at the molecular level should be identified for a better understanding of Cd toxicity in wheat.
- More detailed studies are still required to understand the mechanisms of different amendments in reducing Cd toxicity in wheat.
- Efforts should also be given to identifying and characterize more amendments with Cd decreasing potential and their frequent availability.
- Further studies are needed to reduce Cd toxicity in wheat by combining application of microbes and other amendments such as biochar.
- There is need to evaluate the residual effects of different amendments on Cd uptake by wheat.

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