

Leaf-applied sodium chloride promotes cadmium accumulation in durum wheat grain

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Abstract Cadmium (Cd) accumulation in durum wheat grain is a growing concern. Among the factors affecting Cd accumulation in plants, soil chloride (Cl) concentration plays a critical role. The effect of leaf NaCl application on grain Cd was studied in greenhouse-grown durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) by immersing (10 s) intact flag leaves into Cd and/or NaCl-containing solutions for 14 times during heading and dough stages. Immersing flag leaves in solutions containing increasing amount of Cd resulted in substantial increases in grain Cd concentration. Adding NaCl alone or in combination with the Cd-containing immersion solution promoted accumulation of Cd in the grains, by up to 41%. In contrast, Zn concentrations of grains

were not affected or even decreased by the NaCl treatments. This is likely due to the effect of Cl complexing Cd and reducing positive charge on the metal ion, an effect that is much smaller for Zn. Charge reduction or removal (CdCl_2^0 species) would increase the diffusivity/lipophilicity of Cd and enhance its capability to penetrate the leaf epidermis and across membranes. Of even more significance to human health was the ability of Cl alone to penetrate leaf tissue and mobilize and enhance shoot Cd transfer to grains, yet reducing or not affecting Zn transfer.

Keywords Cadmium · Chloride · Durum wheat · Grain cadmium · Salt

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Introduction

Cadmium (Cd) accumulation in wheat grain is a growing concern, especially in durum wheat. Although the reason is still unclear, durum wheat genotypes accumulate much more Cd in grain than bread wheat genotypes (Meyer et al. 1982; Li et al. 1997; McLaughlin et al. 1998). One of the most promising approaches to reducing Cd accumulation in grain is to breed new genotypes with low Cd concentrations in grain (Clarke et al. 2002). There is a large variation in accumulation of Cd in grain between durum wheat genotypes (Clarke et al. 1997, 2002). Genotypes also differ in

retention of Cd in roots (Florijn and Van Beusichem 1993; Dunbar et al. 2003; Ozturk et al. 2003) and remobilization of Cd from shoot tissues into grain (Hart et al. 1988; Harris and Taylor 2001; Chan and Hale 2004). Existence of such large genetic variation in Cd transport and accumulation in plants can be exploited in breeding programs. Understanding of the mechanisms affecting root uptake, root-to-shoot transport and grain deposition of Cd can significantly improve the success of breeding programs aiming to produce low Cd-accumulating genotypes.

Among the factors affecting root Cd uptake, soil chemical properties play an important role, especially soil pH and organic matter content (Schmidt 2003; Adams et al. 2004; Yanai et al. 2006). Interestingly, in a survey of durum wheat fields varying in soil pH, salinity and other soil factors, grain Cd concentration was very strongly and positively correlated with soil salinity, while the relationship between grain Cd and soil pH was very poor (Norvell et al. 2000; Wu et al. 2002). Soil chloride (Cl) concentration was the parameter that correlated best with grain Cd concentrations. In previous studies with sunflower and potato plants it has been shown that increases in Cl concentration of soils enhanced phytoavailability of Cd to plant roots and thus Cd accumulation in plants (Li et al. 1994; McLaughlin et al. 1994).

The mechanism of the Cl effect on Cd uptake and accumulation in plants is not well understood. Previously, it has been shown that Cd adsorption to soil constituents is reduced by application of Cl, and this effect was ascribed to the formation of soluble Cd–Cl complexes (Bingham et al. 1984). In a study with Swiss chard grown in nutrient solution, Smolders and McLaughlin (1996a) showed that the activity of Cd^{2+} decreased with increased Cl concentration in nutrient solution by forming CdCl_n^{2-n} species, which also reduced Cd uptake by plants as the free Cd^{2+} ion is favored for uptake. However, the reduction in Cd uptake was not as great as the reduction in free Cd^{2+} in solution, suggesting some role for the CdCl_n^{2-n} species in Cd uptake. In subsequent experiments in which Cd^{2+} activity in nutrient solution was held constant (through resin-buffering), increasing Cl concentrations in

solution increased Cd uptake by plants (Smolders and McLaughlin 1996b), strengthening the argument that CdCl_n^{2-n} species play some role in Cd uptake by plants. It is widely believed that chloro-complexation of Cd increases solubility of Cd in soil with concomitant increases in Cd uptake by roots either as a result of improved Cd transport to roots, improved Cd transport through the root apoplast to sites of Cd uptake, or direct uptake of Cd in form of CdCl_n^{2-n} species (Smolders and McLaughlin 1996a; McLaughlin et al. 1997; Lopez-Chuken and Young 2005).

Accordingly, Weggler et al. (2004) showed that shoot Cd concentrations of wheat plants grown in a biosolid-added soil were most closely correlated with CdCl^+ activity in soil solution, while the correlation with the activity of free Cd^{2+} was very weak. Also in a study with different halophyte species, NaCl-induced Cd accumulation in plant shoots correlated with the CdCl_n^{2-n} species much better than with Cd^{2+} , and applying Na_2SO_4 was not effective in increasing Cd concentrations of plants (Lopez-Chuken and Young 2005).

To our knowledge, despite the large number of studies investigating the interaction between Cd and Cl in soil or nutrient solution systems, there has been no study dealing with the effect of the foliar-applied Cl on Cd accumulation in grain. Knowledge of this relationship could contribute to a better understanding of the transport, remobilization and deposition of Cd within plants and also genotypic variation in Cd accumulation. In this study, we aimed to investigate the changes in grain Cd concentration of wheat plants following immersion of intact flag leaves in Cd- and/or NaCl-containing solutions during the grain formation period.

Materials and methods

Plant growth

Seeds of durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) were sown in plastic pots containing 3.6 kg soil and grown under greenhouse conditions in the years 2003 and 2004. The soil used was a Zn-deficient soil containing 0.1 mg DTPA-extractable Zn per kg soil, and therefore

treated with Zn as given below. The soil had a clay texture with a pH of 8.1 and contained 0.7% organic matter and 14% CaCO_3 as measured by using the standard methods described in Jackson (1958). The total concentration of Cd was 0.27 mg kg^{-1} soil and the DTPA-extractable Cd was 0.005 mg kg^{-1} soil determined by using the methods given in Schlichting and Blume (1966) and Lindsay and Norvell (1978), respectively.

About ten seeds were sown in each pot, and after emergence the seedlings were thinned to four seedlings per pot. A basal treatment of 300 mg N kg^{-1} soil as $\text{Ca}(\text{NO}_3)_2$, 100 mg P kg^{-1} soil as KH_2PO_4 , $2.5 \text{ mg Fe kg}^{-1}$ soil as Fe-EDTA and $2.5 \text{ mg Zn kg}^{-1}$ soil as ZnSO_4 was applied to all plants. In some experiments as indicated in the legends of the corresponding experiments, soils were treated with 1 mg Cd kg^{-1} soil in the form of CdSO_4 prior to sowing. Plants were grown until maturation of grains, and at harvest only the spikes were sampled for analysis of the grains. After determination of grain yield per plant, grains were analyzed for Cd, Zn, Mn, Fe and Cu. The concentrations of all metals were measured by inductively coupled argon plasma optical emission spectrometry (Jobin-Yvon, JY138-Ultrace) after digesting the seeds in 65% (w/w) nitric acid with a closed microwave system (Milestone, 1200-Mega).

Leaf application of Cd and NaCl

Leaf application of Cd and NaCl was performed by using intact flag leaves of the main shoot. At the end of the tillering stage, all tillers were removed to eliminate competing sink activity for Cd. After head emergence (nearly after 80 days of growth), whole flag leaves were gently immersed in either Cd- or NaCl-containing solutions for 10 s, once early in the morning and once in the late afternoon. Treatment of the flag leaves was repeated seven times at 4–5 days intervals during the heading and dough stages. To improve wetting of the leaf, the solution contained 100 mg L^{-1} Tween 20. The leaves of the control plants were treated similarly without Cd or NaCl. In the experiment with only leaf-applied Cd, immersion solution contained 0, 0.88 and 8.80 mM Cd in form of $\text{CdCl}_2 \cdot \text{H}_2\text{O}$. In the case of the experiment

with only leaf-applied NaCl, immersion solution contained 0, 50 and 167 mM NaCl . In the experiments investigating the effects of NaCl and different Cd forms on grain Cd concentration, flag leaves were immersed in solutions containing simultaneously both NaCl and Cd-salt (i.e., $\text{CdCl}_2 \cdot \text{H}_2\text{O}$, $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) at a concentration of 8.8 mM Cd and 167 mM NaCl . The pH of these Cd solutions was 5.6, 5.0 and 5.5, respectively.

All experiments were conducted with fourfold replication (pots) by using four plants per replication. The differences among the means were compared by the least significant difference (LSD) test at the 0.05 probability level.

Results

The effect of immersing the leaves in a Cd-containing solution on the grain Cd concentration was studied by using intact flag leaves of wheat plants during the grain development stage. The grain Cd concentration of the plants without Cd treatment of the flag leaves was $50 \text{ } \mu\text{g kg}^{-1}$. Increasing the amount of Cd from 0 to 8.8 mM in the immersing solution in the form of CdCl_2 enhanced Cd concentration of grains by nearly 13-fold (Fig. 1). While the Cd concentrations used in the experiments were high, the immersion time (approximately 10 s) or cycle (seven times with 4–5 days intervals) did not cause significant visible damage on the whole leaf. In the 8.8-mM Cd treatment, only the margins of the treated flag leaves developed some necrotic spots. As the grain yield was not significantly affected by the Cd treatments (Fig. 1), the increases in grain Cd by immersing leaves in the Cd-containing solution were not related to a “concentration effect” that could be caused by reduced grain yield. Similar to the Cd concentrations, the total amount of the grain Cd per plant was also very clearly increased by the immersion of the flag leaves in the Cd-containing solutions.

When the flag leaves were immersed in the NaCl-containing solution (up to 167 mM), there was a significant increase in the grain Cd concentration of the plants grown in a soil treated with 1 mg Cd kg^{-1} soil (Fig. 2). Compared to the H_2O

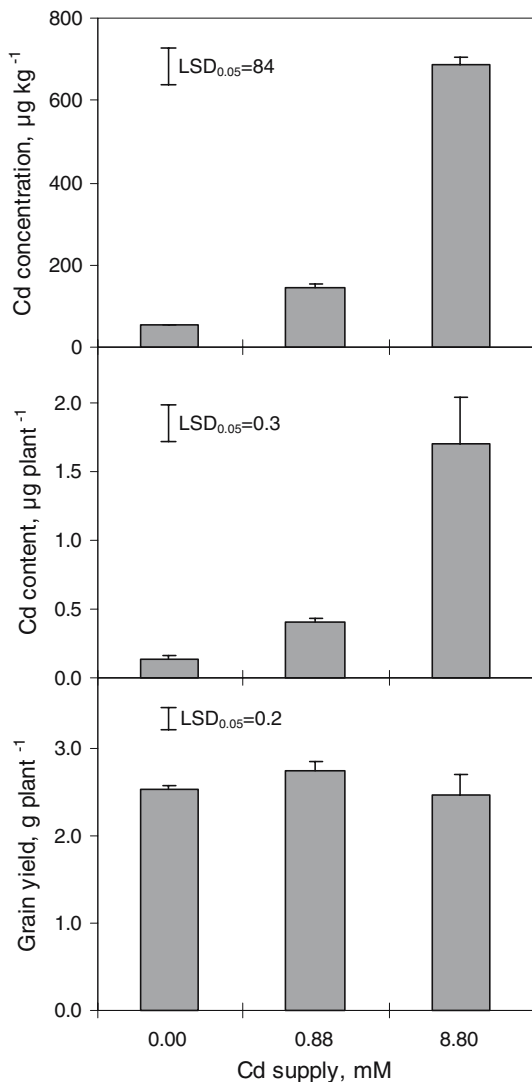


Fig. 1 Effect of leaf-applied $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ on the grain concentration and content of and the grain yield of durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000). The content of Cd was calculated by multiplying grain Cd concentration by the grain yield per plant. Plants were grown in a soil containing 0.005 mg DTPA-extractable Cd per kg soil

treatment, increases in Cd concentrations of grains were 26% with 50 mM NaCl treatment and 41% with 167 mM NaCl treatment (Fig. 2). Grain yield of plants showed a decreasing trend by increasing NaCl application (Table 1), and therefore the NaCl-dependent increases in grain Cd maybe only partly related to a concentration effect. The total amount of Cd in grains per plant

was significantly increased by the 167-mM NaCl treatment, whereas at the 50-mM NaCl treatment, the increase in the total amount of grain Cd was not statistically significant (Fig. 2). In contrast to Cd, both grain Zn concentration and total amount of grain Zn per plant were not affected by immersing the flag leaves in NaCl solution, even significantly decreased at 50 mM NaCl supply (Fig. 2). It seems that the NaCl-dependent increase in grain Cd concentration is specific for Cd.

The effect of NaCl on grain Cd concentration was studied by using three different Cd forms (i.e., $\text{CdCl}_2 \cdot \text{H}_2\text{O}$, $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$). In this experiment, plants were grown in a soil that was not treated with Cd. As found in the experiment presented in Fig. 1, immersing flag leaves in the Cd-containing solution resulted in significant increases in grain Cd concentrations (Table 2). When the flag leaves were immersed in the Cd-containing solution together with 167 mM NaCl, grain Cd concentrations increased significantly with the Cl- and NO_3^- -salts of Cd. On average, NaCl treatment of the leaves resulted in a ~45% increase in grain Cd concentration (Table 2). In the case of the total amount of Cd in grains per plant, NaCl had a greater effect and caused an increase of 60% in the total amount of grain Cd (Table 2). The increase in grain Cd by NaCl was much greater when Cd was added in the immersing solution in the form of CdCl_2 and $\text{Cd}(\text{NO}_3)_2$, and was absent with CdSO_4 (Table 2). Treatment of the leaves with NaCl and with different forms of Cd did not cause any consistent effect on grain yield (Table 2). Thus, the effects of NaCl on grain Cd concentration described in Table 2 were not related to the grain yield. Also in this experiment, in contrast to Cd, the NaCl treatments at each Cd application decreased both the total amount of grain Zn per plant and the concentration of Zn in the grain (Table 2).

Discussion

Immersing flag leaves in the Cd-containing solutions significantly enhanced grain Cd concentration (Fig. 1), indicating that Cd is easily re-translocated from the source (e.g., flag leaves)

Fig. 2 Effect of leaf-applied NaCl on grain concentrations and content of Cd and Zn in durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) grown in a Cd-treated soil at a concentration of 1 mg Cd per kg soil in form of $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. The content of Cd was calculated by multiplying grain Cd concentration with the grain yield per plant

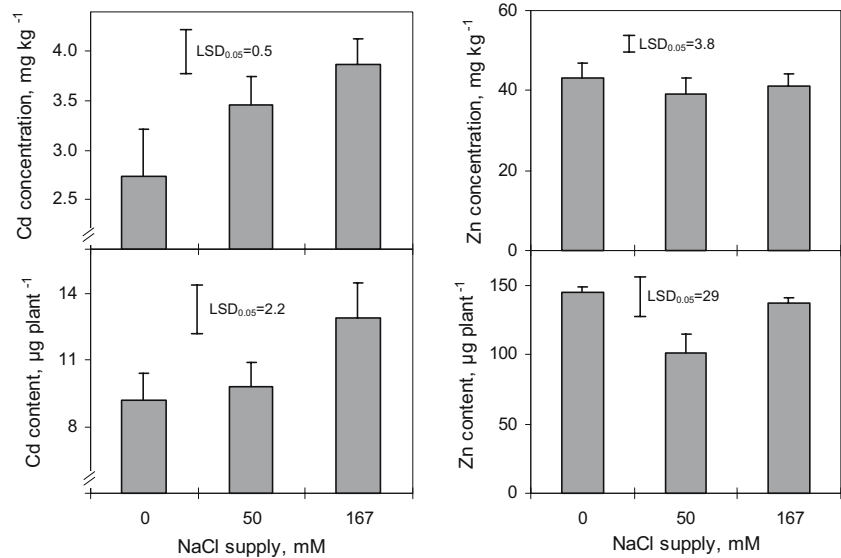


Table 1 Effect of leaf-applied NaCl on grain yield of durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000)

Leaf treatments	Grain yield (g plant ⁻¹)
H ₂ O	3.5 ± 0.7
50 mM NaCl	2.9 ± 0.2
167 mM NaCl	3.2 ± 0.4

into the sink (e.g., grains) organs, most probably via phloem loading. This finding is in good agreement with the results obtained in wheat by Welch et al. (1999), Cakmak et al. (2000a) and Harris and Taylor (2001). The results obtained in

the present study also showed that the translocation of Cd into grain from the flag leaves is promoted by applying NaCl with the Cd-treatment solution. Immersing the flag leaves in the solution containing both Cd and NaCl resulted in significant increases in grain concentration of Cd of wheat plants grown greenhouse conditions (Table 2). The total amount of Cd in grains per plant was also enhanced by leaf-applied NaCl, indicating that NaCl-induced Cd accumulation in grain was not related to the changes in grain yield (e.g., concentration effects) (Fig. 2; Table 2). NaCl-induced Cd accumulation in grains was more pronounced with the $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ and

Table 2 Effect of leaf-applied NaCl and Cd on grain concentrations and contents of Cd and Zn in durum wheat (*Triticum turgidum* L. *durum*, cv. Balcali-2000) grown in unspiked soil

Treatments		Grain cadmium		Grain zinc		Grain yield (g plant ⁻¹)
NaCl	Cd	Concentration (µg kg ⁻¹)	Content (ng plant ⁻¹)	Concentration (mg kg ⁻¹)	Content (µg plant ⁻¹)	
-	-	52 ± 1	130 ± 3	26 ± 2	66 ± 4	2.52 ± 0.05
-	CdCl ₂	341 ± 15	775 ± 236	29 ± 3	66 ± 10	2.27 ± 0.11
-	CdSO ₄	313 ± 83	767 ± 189	25 ± 2	62 ± 6	2.46 ± 0.24
-	Cd(NO ₃) ₂	300 ± 35	676 ± 119	26 ± 4	59 ± 13	2.24 ± 0.28
+	CdCl ₂	545 ± 117	1,504 ± 210	23 ± 1	65 ± 6	2.79 ± 0.10
+	CdSO ₄	341 ± 137	801 ± 260	24 ± 2	58 ± 6	2.40 ± 0.22
+	Cd(NO ₃) ₂	489 ± 135	1,241 ± 274	22 ± 1	57 ± 3	2.57 ± 0.04
LSD _{0.05}		174	363	4	12	0.31

All Cd forms were added at a concentration of 8.8 mM, and +NaCl corresponds to the 167-mM NaCl treatment. Plants were grown in a soil containing 0.005 mg DTPA-extractable Cd per kg soil. Data represents mean of four independent replications. Each replication contained four plants

$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ when compared to the treatment with $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$. The reason that NaCl did not increase grain Cd from the CdSO_4 treatment is not well understood, and needs further investigation. The reason for the stronger effect of NaCl in the case of $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ treatment (Table 2) could be related to the additional Cl ion supplied with the Cd solutions. A similar observation has been made with roots: NaCl-induced Cd uptake by roots was specific to Cl^- anion, and not affected by the SO_4^{2-} or NO_3^- forms of Na (Bingham et al. 1986; Smolders et al. 1996; Lopez-Chuken and Young 2005).

It is well known that Cd and Zn as divalent cations compete for the binding/adsorption sites in cell walls and plasma membranes (e.g., transporter proteins) (Cakmak et al. 2000b; Hart et al. 2002, 2005). Depending on their concentrations, Zn and Cd interfere with their uptake across plasma membranes and transport within plants. Therefore, the NaCl-effect on grain Cd was compared with the grain Zn concentration. In contrast to Cd, leaf-applied NaCl at each Cd treatment did not affect or tended to decrease both the concentration and the total amount of Zn in grains (Table 2; Fig. 2). A key difference between Cd and Zn is the propensity of Cd to form complexes with the Cl ion. For example, using GEOCHEM-PC (Parker et al. 1987), calculations of Cd speciation in the treatment solutions containing 167 mM NaCl predicted that 87% of the Cd in the solutions was complexed with Cl (Table 3), with over 20% of solution Cd being present as the uncharged CdCl_2^0 species. Zinc is not complexed to the same extent as Cd, so that Cl may not be able to mobilize plant Zn to the same extent. Assuming the same Cl concentration in a solution as used in these experiments (167 mM), we calculated that Zn would be predominantly (88%) the free Zn^{2+} ion, with

only 12% complexed by Cl, predominantly present as the ZnCl^+ ion. These results suggest that the effect of the leaf-applied NaCl on the Cd accumulation in grain is highly specific for Cd. Possibly, Cl is involved in Cd uptake across the plasma membranes of the flag leaf cells and/or re-translocation (mobilization) of Cd from the leaves into grains was enhanced by Cl (see below).

To our knowledge, this study is the first to suggest that increasing Cl supply to plants by immersing leaves in NaCl promotes Cd transport from leaves into the grain. A similar phenomenon was also found in soil or nutrient solution systems when roots were exposed to increasing Cl concentrations in the growth medium. Addition of Cl to an unbuffered nutrient solution decreased plant Cd uptake due to a reduction in free Cd^{2+} activity (Smolders and McLaughlin 1996a), but the reduction in free Cd^{2+} ion activity was much greater than the reduction in plant Cd accumulation. When Cd^{2+} activity is buffered (and kept constant) using solution ligands or resins, plant Cd uptake is increased by addition of Cl due to an increase in solution concentration of CdCl_n^{2-n} species (Smolders and McLaughlin 1996a, b). As noted above, Cl forms relatively strong complexes with Cd (compared to Zn) which increase soluble concentrations of Cd in soil solution and increase the phytoavailability of soil Cd to plant roots (Bingham et al. 1984; Li et al. 1994; McLaughlin et al. 1994). Hypotheses to explain this phenomenon are that the transport of Cd across the plasma membranes also takes place in the form of CdCl_n^{2-n} species, as well as free Cd^{2+} ions, or else Cl increases the diffusivity and transport of Cd across the root cell walls and through the apoplast to sites of uptake (Smolders and McLaughlin 1996b; Smolders et al. 1998; McLaughlin 2002).

A plausible hypothesis to explain the results in our study is that a similar chloro-complexation of Cd as found in soil and nutrient solutions can also occur within the plant tissues, leading to increases in solubility and mobility of Cd. This suggestion is supported by the increases in Cd concentration of grains after immersing of the flag leaves into the NaCl-containing solutions (Fig. 2). The results in Fig. 2 indicate an important role of leaf-applied Cl in translocation of root-derived Cd into grain.

Table 3 Predicted distribution of Cd species in the treatment solutions applied to plant leaves, calculated using GEOCHEM-PC (adapted from Parker et al. 1995)

Leaf treatments	Cd species distribution (%)
NaCl + CdCl_2	Cd^{2+} (12.3), CdCl_n^{2-n} (87.7)
NaCl + CdSO_4	Cd^{2+} (13.3), CdCl_n^{2-n} (83.8), CdSO_4 (2.9)
NaCl + $\text{Cd}(\text{NO}_3)_2$	Cd^{2+} (13.7), CdCl_n^{2-n} (86.2), CdNO_3 (0.1)

Transport of leaf-applied Cd from leaves to grain was also enhanced by co-application of NaCl (Table 2). The enhancements in grain Cd concentration by co-application of Cd and NaCl in immersion solution could be associated by formation of soluble Cd–Cl complexes in the immersion solution and thus stimulated penetration of Cd–Cl complexes across the leaf cell membranes as found in roots (Smolders and McLaughlin 1996a; McLaughlin et al. 1997; Lopez-Chuken and Young 2005). A possible role of Na in increasing Cd uptake across membranes cannot be excluded. In future, it is, therefore, important to study also the effects of NaNO_3 or Na_2SO_4 on leaf uptake of Cd. Based on the results obtained with roots, it can, however, be assumed that NaNO_3 or Na_2SO_4 may not be effective in inducing Cd uptake by leaves as found with NaCl. As indicated above, NaCl-induced Cd uptake by roots could not be found with NaNO_3 or Na_2SO_4 (Bingham et al. 1986; Smolders et al. 1998; Lopez-Chuken and Young 2005). In a survey study with 124 paired soil and durum wheat grain samples collected in field, there was a very strong positive correlation between the concentrations of grain Cd and soil soluble Cl (Norvell et al. 2000). This survey study on field-grown durum wheat also indicated that the stimulatory effect of Cl ions on increasing grain Cd is a relevant phenomena occurring under real field conditions. In addition, plants contain relatively high Cl in tissues, such as up to 30 mM Cl in fresh leaf tissue of wheat or up to 120 mM in phloem sap (Marschner 1995; Munns 2002). Such high Cl concentrations could greatly affect solubility and mobility of Cl in plant tissues and significantly contribute to grain Cd concentrations.

Chloride can stimulate solubility and mobility of Cd within shoot and root tissues in different ways. In leaf cells, Cd bound or deposited to cell walls can be mobilized (through complexation) by increasing Cl concentration in the leaf tissue, and consequently an increased pool of soluble Cd could be available in the apoplast for re-translocation into sink organs (e.g., seeds). Reductions in positive charge on the Cd ion as a result of Cl-complexation would reduce retention of Cd by negatively charged cell walls in the apoplast (Clarkson 1968), and there is also the possibility

that penetration of the hydrophobic leaf cuticle was enhanced due to the uncharged nature of the CdCl_2^0 ion present in the high Cl solutions. For example, Gutknecht (1981) showed that diffusion of Hg^{2+} through lipid bilayers was markedly enhanced by complexation to form HgCl_2^0 . The lipophilic properties of an uncharged ion have been shown to increase the absorption of agrochemicals across leaf cuticles by increasing the concentration gradient between the inner cuticle surface and epidermal cells (Schonherr and Riederer 1989). Possibly, similar mechanisms are also involved in phloem loading or re-translocation of Cd from the leaf tissues into the grain. In model studies, it has been shown that Cd strongly binds to negatively charged phospholipids, and the addition of NaCl into the medium containing Cd–lipid complexes promoted release of bound Cd from lipids and resulted in formation of soluble Cd–Cl complexes (Girault et al. 1998). Retention of Cd in roots by adsorption to the cell walls and sequestering into vacuoles is an important factor in reducing Cd transport from roots into shoot (Cieśliński et al. 1996; Chan and Hale 2004; Greger and Löfstedt 2004). Chloro-complexation of Cd in root tissue and the resulting increases in mobility of Cd could favor its transport into shoots. A possible chloro-complexation of Cd in root tissue can promote access of Cd into xylem for transport into shoots, and this can be a further explanation for higher Cd accumulation in shoots and grains of plants grown in saline soils. It would be interesting to study the relevance of Cl⁻ in reducing Cd sequestration in vacuoles and binding of Cd to root cell walls thus stimulating Cd loading into xylem for shoot transport.

There is increasing evidence in the scientific literature showing that remobilization of Cd from stem or leaf tissues play an important role in grain Cd accumulation (Hart et al. 1998; Harris and Taylor 2001; Chan and Hale 2004). It is speculated that variation in Cd remobilization from shoot tissues could be an important factor contributing to genetic differences in grain Cd accumulation. Based on the results presented in this paper, it can be assumed that remobilization of Cd deposited in stem or other plant tissues could be promoted in the presence

of Cl by formation of mobile CdCl_n^{2-n} complexes. It is, therefore, important to choose carefully and monitor Cl concentrations of plants and growth mediums in the studies dealing with uptake and transport of Cd. This would be especially important when different genotypes are used which may have different Cl concentrations in roots and shoots. Future research should focus on the role of Cl in transport of Cd within the plant tissues and remobilization of Cd deposited in the cell walls of root and shoot tissues.

On a more practical front, our data also indicate that irrigation of crops with saline waters containing high Cl concentrations may not only mobilize Cd from soil, but also increase the translocation of Cd from shoots to grains. The differential effect of Cl in remobilizing plant Cd transfer to grains, but not Zn, creates an added hazard for humans as bioavailability of food Cd is highly dependent on the Cd:Zn ratio.

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