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ORIGINAL PAPER

Cadmium Reduction in Spring Wheat: Root Exudate Composition Affects Cd Partitioning Between Roots and Shoots

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Abstract

Cadmium (Cd) in cereals is one of the major sources of Cd intake by human diets, and solutions to reduce Cd concentrations in wheat still need to be developed. Plant breeding, by selecting low-Cd varieties, can be an important tool to reduce Cd in crops. Knowing the genotypic variation in Cd accumulation and furthering our understanding of the impact of root exudates composition on Cd accumulation in crops may provide valuable information for plant breeding. In this study, we selected nine spring wheat varieties and analysed the accumulation and distribution of Cd in shoots, roots, root surfaces and kernels in relation to their qualitative and quantitative composition of root exudates, determined by ^{1}H-NMR (Proton Nuclear Magnetic Resonance). Results showed that the Cd concentration in shoots at an early stage could be used as a predictor for Cd concentration in kernels. Total Cd uptake was not correlated to the mobility of Cd in the rhizosphere, but total Cd was negatively correlated to Cd adsorbed at the root surface. Furthermore, (i) exudation of organic acids (primarily succinate and acetate) increased Cd concentration in shoots, and (ii) exudation of nucleosides, DNA (deoxyribonucleic acid) degradation products, increased Cd adsorption at the root surface. Therefore, root exudates composition should be taken into account when selecting for low-Cd wheat traits.

Keywords ^{1}H-NMR · Organic acids · *Triticum aestivum* · Heavy metal · Genotypic variation · Extracellular DNA

Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
EDTA	Ethylenediaminetetraacetic acid
exDNA	Extracellular deoxyribonucleic acid
ICP-MS	Inductively coupled plasma mass spectrometry
RE	Root exudate

Dedicated to the memory of Holger Kirchmann.

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1 Introduction

Cadmium (Cd) is classified as a human carcinogen (IARC 2012). In soils, Cd can originate from anthropic (industry and agriculture) or natural (geogenic origin, volcanic emissions) sources (Singh and McLaughlin 1999). Its relative high mobility in soils leads to an easy plant uptake. Consequently, cereals and vegetables are the major sources of Cd intake in diets of non-smokers. Due to the ability to accumulate in the body and its high toxicity even at low intake, human health can be negatively affected (kidney failure and increased risk of cancer) (EFSA 2009). Yet, agronomic measures to reduce Cd levels in crops are still lacking and management improvement is therefore required.

There is a natural variation in uptake and distribution of essential and non-essential trace elements among crop species but also among varieties within the same species. Previous research showed that Cd accumulation in wheat grains varies among cultivars (Greger and Landberg 2008; Greger and Löfstedt 2004; Kubo et al. 2008; Lu et al. 2020; Rabnawaz et al. 2017; Stolt et al. 2006). Plant breeding can therefore be an important tool to reduce Cd concentrations in wheat. Knowing the genotypic variation and the physiological processes causing Cd accumulation in crops may help breeders targeting traits involved in low Cd uptake. For example, composition and quantity of root exudates can affect heavy metal uptake. Indeed, single compound or a subgroup of exudates can play a significant role for Cd bioavailability, uptake and translocation in various plant species (Chen et al. 2020; Liu et al. 2007; Luo et al. 2017; Qin et al. 2021; Xin et al. 2015). Identification and quantification of root exudates as well as their genetic background can therefore be a useful tool to exploit a crop's potential for heavy metal uptake (Kuijken et al. 2015).

Differences in grain Cd accumulation among wheat varieties could be due to their variability in Cd allocation in different plant parts including grains; to an altered Cd uptake ability of roots; and/or to a higher capacity to mobilise it from the soil. Greger and Landberg (2008) showed that high-Cd wheat varieties did not take up more Cd than low-Cd cultivars when provided in a nutrient solution. They concluded that the differences in Cd uptake are related to a different ability to mobilise Cd in the rhizosphere rather than to a different root absorption capacity. Indeed, it is known that by releasing root exudates into the rhizosphere, plants can modify the biogeochemistry of the soil and change the bioavailability of nutrients and contaminants (Badri and Vivanco 2009; Dong et al. 2007). Hence, a higher Cd accumulation in the plant may be the result of a higher release of Cd from soil particles by root exudates, and thereby a higher concentration of Cd in the soil solution. Root exudates constitute a wide range of organic compounds including carbohydrates, organic acids, fatty acids and enzymes (Badri and Vivanco 2009). Previous studies highlighted that a higher release of organic acids (Dong et al. 2007; Zhu et al. 2011) and phytosiderophores (Hill et al. 2002) reduced Cd bioavailability and thereby uptake by plants. Moreover, in the plant, chelation with some ligands such as histidine and citrate appears to route metals to the xylem, promoting root to shoot transport (Clemens et al. 2002).

In the present study, we aimed at evaluating whether various spring wheat varieties and their root exudation characteristics affect Cd mobility in the rhizosphere and consequently Cd uptake and allocation in the crop. We hypothesised that (i) 'high Cd cultivars' mobilise more Cd from soil particles, and (ii) 'low Cd cultivars' present a higher ability to exclude Cd from the roots, and (iii) higher exudation of amino acids and organic acids increases Cd uptake and translocation in wheat.

2 Material and Method

2.1 Soil

The soil was sampled in an agricultural field in Skåne, south of Sweden. A soil presenting naturally high Cd concentration (0.98 mg kg^{-1}), leading to high Cd concentration in wheat was sought; the sampling area was therefore selected according to the results from Söderström and Eriksson (2013). Soils from this area have organic matter content around 3–4% and pH of 6.5 (Eriksson 1990). The air-dried soil was sieved to 2 mm and mixed with sand at 1:1 weigh ratio resulting in a Cd concentration of the soil and sand mixture of 0.49 mg kg^{-1}.

2.2 Plant Material and Growth

Nine spring wheat genotypes were used as they vary in roots and shoots traits as well as in nutrient accumulation (Liu et al 2021). Seeds from eight genotypes were obtained from Lantmännen: 'KWS Alderon' ('Alderon', Germany, KWS W185), 'Bjarne' (Sweden, NK 97,520), 'Boett' (Sweden, SW 71,034), 'Dacke' (Sweden, WW 26,267), 'Diskett' (Sweden, SW 45,456), 'Happy' (Sweden, SW 91,003), 'Quarna' (Switzerland, CH 21,112,283) and 'Rohan' (Sweden, SW 01,198). The last genotype, 'Dala LR', a landrace originating in Dalarna (Sweden) was supplied by local farmers. Grains were germinated for 3 days on a wet filter paper in the dark at 21 °C. Seedlings were transplanted to 50-ml tubes (one seedling per tube) filled with the soil:sand mixture. Plants were grown for 21 days with 12-h light, 24 °C during the day and 19 °C during the night and constant humidity (60%) in a growing cabinet (CLF Plant Climatics Modell SE41-E2, Germany). A full fertiliser ('Blomstra') was used, and fertiliser solutions were added at transplanting, 7 and 15 days thereafter. The total amount of nutrients added to 1 g of soil:sand mixture was: 382 µg N g^{-1}, 75 µg P g^{-1}, 322 µg K g^{-1}, 30 µg S g^{-1}, 22 µg Ca g^{-1}, 30 µg Mg g^{-1}, 2.6 µg Fe g^{-1}, 1.5 µg Mn g^{-1}, 0.75 µg B g^{-1}, 0.23 µg Zn g^{-1}, 0.11 µg Cu g^{-1} and 0.03 µg Mo g^{-1}.

For determination of Cd contents, additional wheat kernels were obtained from the same nine genotypes grown in a field experiment situated in Säby (Sweden) in 2019 (Weih et al.;, 2021). Standard fertilisation rates were applied after sowing (140 kg N ha^{-1}, 24 kg P ha^{-1}, 46 kg K ha^{-1}). In the field experiment, all varieties were grown in 88 m^2 plots (distributed as 22 m^2 parcels on four blocks). Wheat seeds were sown on the 23rd of April 2019. Seed rates were 550 seeds m^2, as common in the region. Final harvest of the central plot area (6 m × 2 m) was done on the 23rd of August 2019 with a combine harvester to assess agronomic grain yield. Further details on the experimental conditions can be found in Weih et al. (2021) and Liu et al. (2022). Kernels analysed were the result from pooled samples from the four blocks. Results presented are the average of four analytical replicates.

2.3 Root Exudate Sampling and Analysis by H^1-NMR (Proton Nuclear Magnetic Resonance)

Root exudates were sampled from the seedlings grown for 21 days in the growing cabinet, adopting a soil-hydroponic-hybrid method proposed by Oburger and Jones (2018): After a 21-day growing period, wheat plants were sampled after 5 to 7 h of light exposure between 10 and 12 a.m. in the morning. The root system was carefully removed from the soil to avoid root damage. Roots were then thoroughly washed and treated with deionised water. Plant roots were soaked in H_2O for 30 min to remove molecules released due to root damage. After 30 min, the root system was rinsed and the solution was replaced by fresh H_2O. Plants were then placed in a growing cabinet for 2 h (CLF Plant Climatics Modell SE41-E2, Germany) in order to collect root exudates. After collection, the solution was filtered through 0.2-µm mesh and immediately placed at − 20 °C prior freeze-drying.

Freeze-dried root exudates were dissolved in 500 µL ultra-pure H_2O, before analysis by H^1-NMR. A volume of 150 µL phosphate buffer (0.4 mol/L, pH 7.0) and 45 µL D_2O were added to 375 µL of re-dissolved root exudates samples. To ensure quantitative measurements of metabolites, 30 µL of sodium-3-(trimethylsilyl)-2,2,3,3-tetradeuteriopropionate (5.8 mmol/L) (Cambridge Isotope Laboratories, Andover, MA), used as an internal standard, was also added (Moazzami et al. 2012).

The mixture was used for ^{1}H-NMR analysis with a Bruker Avance III spectrometer operating at 600 MHz equipped with a cryogenically cooled probe and autosampler. ^{1}H-NMR spectra were acquired using zgesgp pulse sequence (Bruker Spectrospin Ltd) at 25 ℃, with 128 scans and 65,356 data points over a spectral width of 17,942.58 Hz (acquisition time 1.83 s, relaxation delay 4 s) (Röhnisch et al. 2018).

The ^{1}H-NMR signals were identified and quantified using the NMR Suite 7.1 library (ChenomX Inc, Edmonton, Canada). Peak size for quantification was made manually following the same order and reference peak per compound for all the spectra (see supplemental data S1).

Compound flux was calculated as follows:

$$\text{root exudates flux} = \frac{C \times RV \times d}{R} \div h$$

where C is the concentrations measured with ^{1}H-NMR in mM, RV is the volume in which the freeze dried root exudates where resolubilised, in millilitres (ml), d is the dilution factor as the resolubilised sample was diluted in phosphate buffer, D_2O and internal standard solution.

R correspond to the root dry weight in g, and h the sampling time of the root exudates, in hours.

2.4 Determination of Cadmium Concentration Adsorbed at the Root Surface

After root exudate collection, plant roots were transferred in about 35 ml (volume exactly determined by weighing) of 20 mM Na_2EDTA solution for 30 min. The solution was then filtrated at 0.2 µm and stored at + 4 ℃ before Cd analysis by inductively coupled plasma-mass spectrometry (ICP-MS, Perkin Elmer, NexIon 350d). A performance check of the instrument was done prior each analytical sequence according to the manufacturer recommendations. The calibration standards (0.1 to 50 µg L^{-1}) were matrix matched to the sample matrix, and ^{115}In was used as an internal standard. Detected Cd concentrations were corrected for procedural blanks. The results are expressed in µg g^{-1} dry weight (DW) of roots.

2.5 Determination of Cadmium Concentrations in Wheat

Shoots and roots from the 21-day old plants were washed and separated prior freeze-drying. Dry shoots and roots systems were placed in a Teflon vessel and weighed before acid digestion using 65% HNO_3 and a microwave digestion system (Ethos easy, Milestone srl, Italy) following the EPA 3052 guidelines (U.S Environmental Protection Agency 1996). Approximately 1 g of kernel was weighed and digested using the same method (Potočnik et al. 2021; Zhang et al. 2019). The mineralisation products were filtered with a 0.45-µm mesh and stored at + 4 ℃ before Cd measurement. Cadmium was measured in digestate (diluted 1:10 times with ultrapure water) using ICP-MS (PerkinElmer Nexion 350D) as indicated above. Quality assurance and accuracy of the digestion and analytical method were checked using a plant certified reference material (ERM-CD281 Rye grass, European Reference Materials) with accuracy within 100 ± 10%.

The translocation factor (TF), i.e. root to shoot allocation ratio was determined as follows:

$$TF = \frac{Cd_{\text{Shoots}}}{Cd_{\text{roots}}}$$

With Cd_{shoots}, the concentration of Cd measured in shoots in µg.g^{-1}; and Cd_{roots} the concentration of Cd measured in roots in µg g^{-1};

Plant Cd uptake was calculated as follows:

$$Cd_{\text{uptake}} = \left(Cd_{\text{shoots}} \times m_{\text{shoots}}\right) + \left(Cd_{\text{roots}} \times m_{\text{roots}}\right)$$

With Cd_{shoots} and Cd_{roots}, the concentration of Cd measured in shoots and roots (µg g^{-1}) respectively; and m_{shoots} and m_{roots} biomass dry weight of shoots and roots (g), respectively.

2.6 Determination of Mobile Cadmium Concentration in Soil

A 0.05 M ethylenediaminetetraacetic acid (EDTA) (pH = 7.00 ± 0.05) solution was used as an extractant and prepared following the protocol of the Community Bureau of Reference (CBR) (Quevauviller 1998). A volume of EDTA corresponding to a soil:solution ratio of 1:10 w/v was added to fresh soil sample. The mixture was stirred at room temperature on an orbital shaker at 125 rpm for 1 h. The tubes were then centrifuged for 10 min at 8000 rpm, and the supernatants were collected and filtered to 0.45 µm. The resulting solutions were stored at + 4 °C until Cd analysis by ICP-MS. The soil pellet was air dried for a week and dry soil weight was used for calculation.

2.7 Statistical Analysis

All statistical analyses and graphical presentations were performed using R software (R Core team 2019), except Fig. 3, made with Microsoft Excel (Microsoft corporation 2016).

Kruskal Wallis test for average differences and posthoc Dunn test using the Benjamini-Hochberg (BH) method for *p* value correction for multiple comparisons were performed using the function *dunn.test* from the package "dunn.test" (Dinno 2017).

Spearman correlations between groups were performed with the *statsby* function from the package "psych" (Revelle 2019) and the *p.adjust* function, from the package 'stats' (R Core team 2019) using the Benjamini-Hochberg (BH) method for *p* value correction for multiple comparisons. The linear models were performed using the function *lm* from the package "stats" (R Core team 2019).

3 Results

3.1 Cd Accumulation and Distribution in the Different Genotypes

No significant differences were found between the nine genotypes for Cd concentrations in shoots which ranged between 0.47 ± 0.04 (mean ± SE, $n = 4$) and 0.66 ± 0.09 µg g^{-1} DW (Fig. 1A). However, significant Cd differences (Kruskal–Wallis test, $p < 0.05$) were highlighted for roots (Fig. 1B) and Cd adsorbed onto root surfaces concentrations (Fig. 1C), as well as for Cd contents in the entire plant (Cd in shoots + Cd in roots) (Fig. 1D), corresponding to the total Cd plant uptake. The root concentrations ranged between 1.40 ± 0.04 for 'Boett' and 2.66 ± 0.26 µg g^{-1} DW for 'Diskett'; the concentrations of Cd adsorbed onto the root surface ranged between 0.46 ± 0.1 for 'Dacke' and 1.10 ± 0.03 µg g^{-1} DW for 'Alderon'. Average Cd content ranged from 0.83 ± 0.10 to 1.56 ± 0.31 µg Cd $plant^{-1}$ for 'Bjarne' and 'Diskett', respectively.

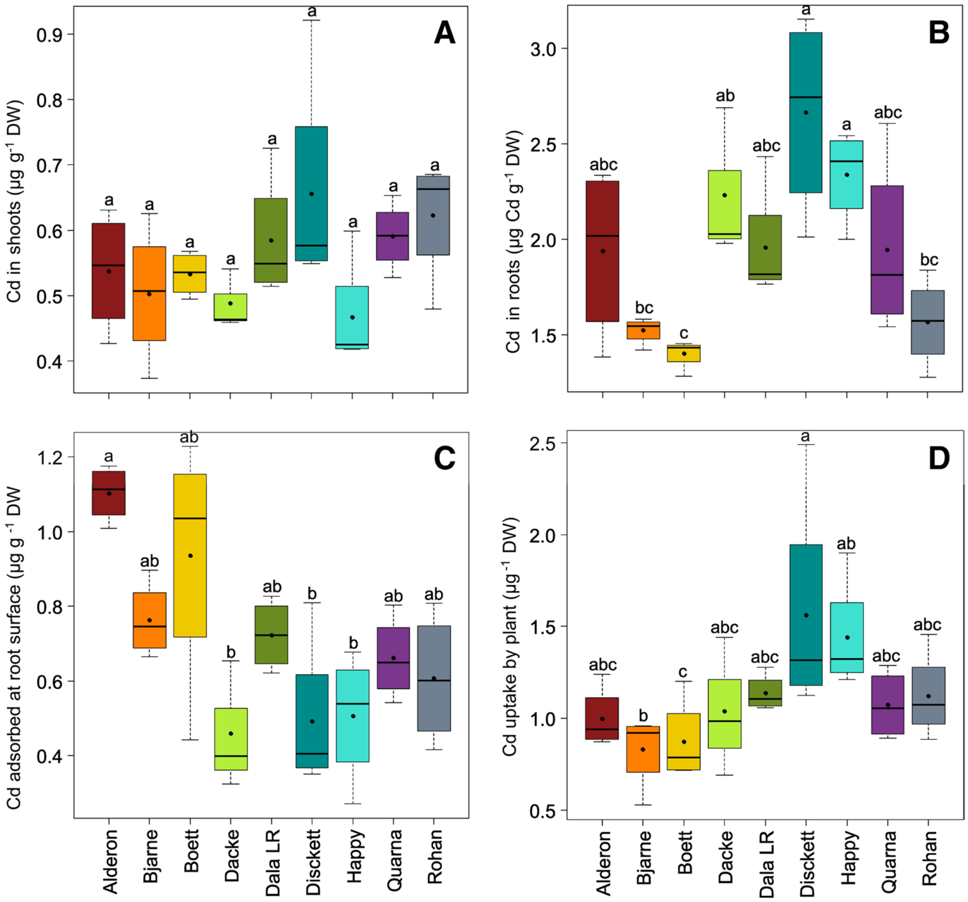

Fig. 1 Boxplot representing the concentrations of Cd in shoots (**A**), roots (**B**), and adsorbed onto root surfaces (**C**) in µg g^{-1} DW. Cadmium uptake in µg Cd per plant is shown in (**D**). The black dots represent the average concentrations ($n = 4$). Statistically significant differences (Kruskal–Wallis with Dunn's posthoc test, significant p values < 0.05) are represented by different letters above each boxplot

Concentrations in the kernel (Table 1) from the same nine genotypes grown to maturity in the field showed significant differences with the lowest values for ‘Happy’ (63.9 ± 4.6 ng Cd g^{-1}) and the highest for ‘Quarna’ (115 ± 6.6 ng Cd g^{-1}) (Table 1). The Swedish criterion for quality-assured low Cd level products in grain is currently 100 ng Cd g^{-1} for spring wheat (Svenskt Sigill 2011). In the kernel samples analysed, only ‘Quarna’s kernels had a Cd concentration higher than this limit and ‘Diskett’, ‘Dala LR’ and ‘Rohan’ were close with concentrations higher than 90 ng Cd g^{-1}.

Several significant correlations were highlighted between mean Cd concentrations in the different plant tissues, giving informations about Cd allocation pattern in wheat. The results highlighted that Cd concentration in the kernels was correlated to Cd concentration in the shoots ($\rho = 0.77$, $p < 0.05$) (Table 2). Moreover, adsorption of Cd at root surfaces appeared negatively correlated with Cd present in roots ($\rho = -0.74$, $p < 0.1$) and Cd taken up by the plants ($\rho = -0.68$, $p < 0.1$). For all genotypes, Cd was mainly stored in roots amounting between 43 and 65% of total crop Cd with ‘Dacke’, ‘Diskett’ and ‘Happy’ being over 60% (Fig. 2). The fraction of Cd adsorbed at the root surface ranged from 9 to 31%. ‘Alderon’ and ‘Boett’ showed the highest Cd exclusion with more than 25% of total Cd present at the root surface. The fraction of Cd stored in shoots ranged from 25 to 40%, with ‘Bjarne’, ‘Quarna’, ‘Rohan’ and ‘Dala LR’ accumulating more than 35% of the total Cd content in shoots.

Table 1 Cd concentrations in kernels. Results are the mean (±SD) of four analytical replicates. Statistically significant differences (Kruskal–Wallis with Dunn's post hoc test, significant p values < 0.05) are represented by different letters

Genotype	Cd in kernels (ng g^{-1})
Alderon	84.7 ± 10.0 cde
Bjarne	75.7 ± 4.6 ef
Boett	95.0 ± 8 .1 b
Dacke	83.7 ± 4.6 de
Diskett	99.1 ± 6.1 ab
Happy	63.9 ± 4.6 f
Quarna	115.0 ± 6.6 a
Rohan	90.9 ± 7.3 bcd
Dala LR	93.4 ± 7.6 bc

3.2 Effects of Cd Mobility and Root Exclusion on Cd Uptake

Rhizospheric mobile Cd, here defined as the EDTA-extractable Cd in rhizosphere soil, was significantly different between the genotypes (Kruskal–Wallis test, $p < 0.01$) varying between 0.17 ± 0.012 and 0.21 ± 0.005 µg Cd g^{-1} dry soil (Fig. 3A). No correlation was observed between the ability of genotypes to mobilise Cd in the rhizosphere and Cd uptake (Spearman, $\rho > 0.05$). However, Cd uptake was significantly correlated to the concentration of Cd adsorbed at the root surface (Fig. 3B, Spearman, $\rho < 0.05$), with lower uptake for the genotypes with higher Cd exclusion capacities.

3.3 Composition of Root Exudates

Forty-five different root exudates (RE) compounds were identified via ^{1}H-NMR. Concentrations of main compounds identified are shown in supplementary data (Supplementary Table S2.1 to S2.5). The four main chemical categories were sugars, amino acids, organic acids as well as nucleic acids

Table 2 Spearman's correlation among Cd concentrations, Cd uptake and the translocation factor (TF). The upper part shows the correlation coefficients (black bold text, significant at $p < 0.05$; grey bold text: significant at $p < 0.1$), and the lower part p values adjusted for multiple correlation with Benjamini–Hochberg method

	Cd_{Kernel}	Cd_{Shoots}	Cd_{Roots}	$Cd_{Adsorbed}$	Cd_{Uptake}	TF
Cd_{Kernel}		**0.77**	0.01	0.02	0.19	0.32
Cd_{Shoots}	0.05		0.10	-0.10	0.39	0.35
Cd_{Roots}	0.97	0.91		**-0.74**	**0.84**	**-0.85**
$Cd_{Adsorbed}$	0.97	0.91	0.06		**-0.68**	0.48
Cd_{Uptake}	0.81	0.47	0.02	0.10		-0.50
TF	0.56	0.54	0.02	0.33	0.33	

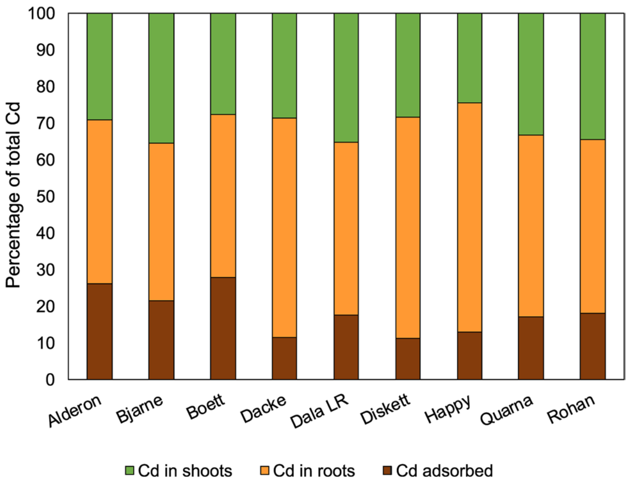

Fig. 2 Cd distribution in shoots (top: green colour), roots (middle: orange colour) and adsorbed at the root surface (bottom: brown colour) as a percentage of the total Cd amount in plants (sum of Cd in shoots, roots and adsorbed at the root surface)

and their derivatives representing, on average, 50%, 15%, 25% and 1.5% of the amount of total RE identified, respectively (Table 3).

3.4 Correlation Between Root Exudates and Cd Concentrations in Wheat Varieties

Trends were found between Cd concentrations in shoots and the exudation of succinate and acetate (Fig. 4A and B) (Spearman's correlations: $\rho = 0.60$ and 0.62, respectively, $p < 0.1$). Correlations became highly significant when 'Diskett' was discarded (Spearman's correlations: $\rho = 1.0$ and 0.97 for acetate and succinate respectively, $p < 0.0001$).

Furthermore, the Cd concentration adsorbed at the root surface, i.e. excluded from root uptake, was significantly correlated with the exudation of deoxyribonucleosides (Fig. 4D, sum of exudations of thymidine, 2′deoxyadenosine, 2′deoxyguanosine) (Spearman's correlation $\rho = 0.90$, $p < 0.05$).

4 Discussion

This study focuses on how root exudates composition can influence Cd uptake and translocation by spring wheat. Using nine different genotypes with various Cd uptake capacities, root exudate composition was quantified and correlated with concentrations as well as Cd distribution in the various soil–plant compartments. Studies focusing on the role of root exudates for trace metal and metalloids detoxification are often performed under hydroponic conditions (Fu et al. 2018; Luo et al. 2017; Rossi et al. 2018; Taddei et al. 2002; Zhao et al. 2016),which do not represent realistic conditions for root exudation in soil. To get closer to more realistic composition and concentration of root exudates, we evaluated plant-soil interactions by adopting a soil-hydroponic-hybrid method as defined by Oburger and Jones (2018).

The first hypothesis was that 'high Cd cultivars' can mobilise more Cd from soil. Soil pH is known to be a primary factor influencing plant availability of Cd (Eriksson 1990; Kicińska et al. 2022) and very important for Cd uptake by wheat (Liu et al. 2015). Moreover, it has been suggested that differences in Cd concentrations in wheat cultivars were related to their ability to mobilise Cd from the soil (Greger and Landberg 2008). Our results did not corroborate these findings showing no correlation between mobile Cd in rhizospheric soil and Cd uptake by wheat cultivars. These results suggest that (i) either the soil extraction method used was not the most representative of bioavailable Cd or (ii) other processes than the solubilisation of Cd from the soil are involved in the genotypic difference, for example, intrinsic plant physiology processes such as

Fig. 3 Cadmium taken up by plant (µg) as a function of (**A**) the concentration of Cd mobile in the rhizosphere (µg g^{-1} DW) and (**B**) the concentration of Cd adsorbed at the root surface (µg g^{-1} DW). Value of ρ corresponds to the Spearman correlation coefficient (*significant at $p < 0.05$; ns, non-significant ($p > 0.1$). R^2 correspond to the coefficient of determination for the model applied (dotted lines)

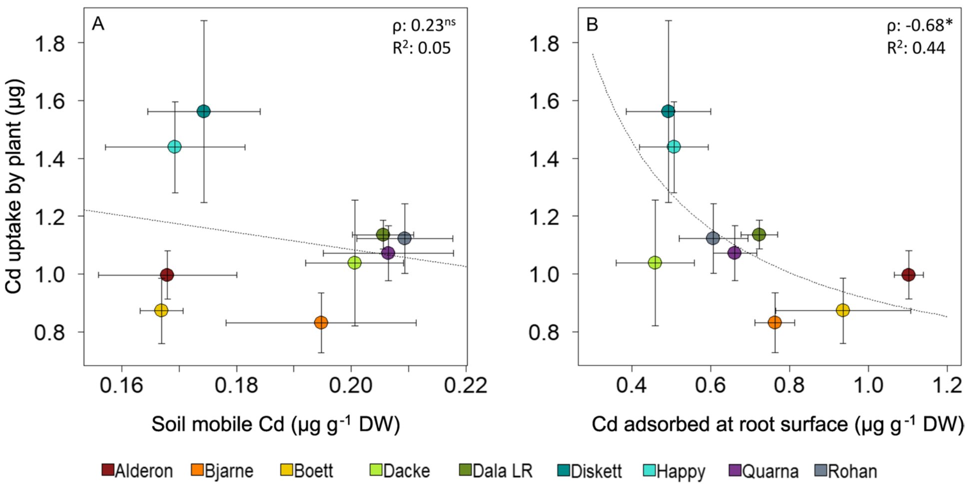

Table 3 Composition of root exudates separated into different compound classes

Compound class	% of identified root exudates	Type of compound identified
Sugars	38–65	Fructose, galactose, glucose, maltose, sucrose, xylitol
Amino acids	9–22	4-Aminobutyrate, alanine, alloisoleucine, asparagine, aspartate, histidine, isoleucine, methionine, phenylalanine, proline, tryptophan, tyrosine, valine
Organic acids	16–39	Acetate, cinnamate, citrate, formate, fumarate, lactate, malate, succinate
Nucleic acids and derivatives	0.5–2	2′-Deoxyadenosine, 2′-deoxyguanosine, 2′-deoxyuridine, adenine, adenosine, cytidine, guanosine, thymidine, thymine, uridine
Others	4–9	3-hydroxybutyrate, betaine, creatine phosphate, galactarate, N-acetyltyrosine, O-phosphocholine, oxypurinol, glycerophosphocholine

the type of membrane metal transporter (Guo et al. 2022) or root morphology and anatomy (Huang et al. 2019).

Another hypothesis was that ‘low Cd cultivars’ have a higher ability to exclude Cd from roots. The negative correlation between Cd uptake and the concentration of Cd adsorbed at the root surface highlighted that Cd adsorbed on the root surface cannot be taken up. Therefore, a higher root adsorption capacity may result in a lower Cd uptake. The non-linear shape of the relation between Cd adsorption and plant uptake indicates that some Cd is taken up by wheat cultivars irrespectively of the exclusion capacity of the various wheat cultivars. These observations are in line with results of Cheng et al. (2021) which showed that an increase of Cd adsorbed at root surface is accompanied by a decrease of Cd accumulation in wheat cultivated with a strain of metal-resistant bacteria. However, it is in contradiction with results showing that high-Cd-accumulating winter and durum wheat genotypes adsorbed more Cd on the root surface than low-Cd-accumulating genotypes or isolines (Adeniji et al. 2010; Xiao et al. 2020). Exclusion mechanisms may differ between different types of wheat (spring, durum and winter) but the differences observed between the studies could also be explained by desorption methods, Cd exposure period and cultivation media, which differed among studies.

The strong positive correlation between the concentration of deoxyribonucleosides (thymidine, deoxyadenosine, deoxyguanosine) in root exudates and the concentration of Cd adsorbed at the root surface support the mechanism that Cd adsorption hinders plant uptake (see above). This observation suggests that the exudation of deoxyribonucleosides may contribute to the formation of a ‘DNA trap’ formed by root border cells. One may assume that the main origin of deoxyribonucleosides, i.e. the skeleton of deoxyribonucleic acid (DNA) constituents, is derived from lysed cells. However, there is evidence that extracellular DNA (exDNA) can be actively released from living roots (Nagler et al. 2018). Root border cells, also called ‘sloughed root cap cells’ are able to release exDNA into the mucilage layer (Hawes et al.

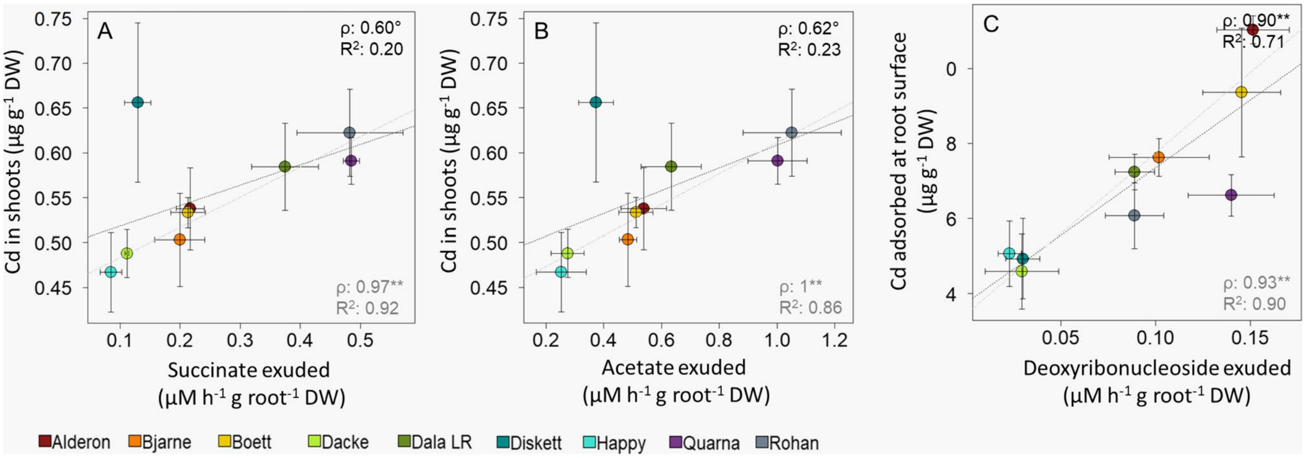

Fig. 4 Concentrations of Cd in the shoots as a function of succinate exudation (**A**) and acetate exudation (**B**). Concentration of Cd adsorbed at the root surface as a function of deoxyribonucleosides exudation (**C**). All exudation concentrations are given in µM h^{-1} g $root^{-1}$ DW. The lines represent the results of the linear model. Black dotted line and the black values for ρ (Spearman's correlation coefficient) and R^2 are the results for all the genotypes. The grey dotted line and grey values of ρ and R^2 are the results when Diskett was discarded. **Significant at $p<0.01$, °Significant at $p<0.1$

2016). In fact, extracellular DNA has been shown to have a protective role against biotic and abiotic stress, including contaminants such as aluminium, arsenic (Hawes et al. 2016) and lead (Huskey et al. 2019, 2018). Moreover, it is known that mucilage can create a continuum between the soil and roots promoting water uptake (Ahmed et al. 2014). In the present study, deoxyribonucleoside exudation and Cd adsorption at the root surface were highly and positively correlated to water uptake rates (correlation coefficient $r=0.97$, $p<0.0001$ and $\rho=0.89$, $p<0.01$, respectively; results not shown). This may indicate the involvement of mucilage in the exclusion of Cd from crop uptake. Further research relating to the release of deoxyribonucleosides from root border cells and Cd adsorption would be needed to confirm whether this mechanism is in place. Here, experimentations similar to the ones conducted by Huskey et al. (2019) for Pb could be adopted. Cadmium trapping by root border cells could be evidenced by applying a Cd staining agent to a root tip immersed in a Cd solution and exDNA role revealed by addition of nuclease.

The third hypothesis was that root exudate compounds are involved in Cd uptake, transport and distribution in the crop. Comparison of root exudates concentration with other studies can be challenging due to the use of different species, but also due to different collection and analyses methods. However, several studies highlighted flux of organic acids and amino acids in the same order of magnitude in wheat, barley, rice (Fan et al. 2001), fava bean (Lv et al. 2020), hot pepper (Xin et al. 2015) and tomato (Zhu et al. 2011). Therefore, the relationships between the 45 compounds identified and the Cd content in spring wheat plants were investigated. Our results showed that the composition of root exudates had an influence on Cd distribution in the plant with a higher translocation of Cd to the shoots with higher acetate and succinate exudation. It has been suggested that root to shoot transport of metals is increasing when metal-chelate complexes, such as Cd-citrate, are involved (Senden et al. 1992). Studies showed that low molecular weight organic acids are involved in root to shoot translocation of metal ions in the form of complexes. Citrate seemed to be particularly involved in metal transport in the xylem (Kutrowska and Szelag 2014; Li et al. 2019). Chen et al. (2020) highlighted that addition of root exudates with 100 mmol L^{-1} of citric acid promoted Cd transfer from roots to shoots. In the present study, the concentrations of Cd in shoots of wheat plants were positively correlated to the concentrations of organic acids, particularly of succinate and acetate. The two organic acids were also positively correlated with translocation factors ρ:0.73, $p<0.05$ for acetate and ρ:0.67, $p<0.05$ for succinate (Spearman's correlation; results not shown). Assuming that Cd-organic acid complexes can be directly taken up in the rhizosphere and transported within the plants is supporting the idea of their involvement in Cd transport in the xylem. Cieśliński et al. (1998) found higher acetic and succinic acid concentrations in the rhizosphere of high Cd durum wheat genotype (Kyle) compared to the non-accumulating genotype (Arcola). They suggested that the involvement of these two organic acids could be extended to spring and durum wheat. Extrapolating the effect of succinate and acetate exudation to spring wheat was supported by our data except for 'Diskett'. Compared with the other genotypes, 'Diskett' showed relative higher Cd concentration in shoots in relation to exuded succinate and acetate concentrations. For this variety, other unknown mechanisms may be involved in the Cd transport from roots to shoots.

Finally, a significant positive correlation was found between Cd concentrations in 3-week old plant shoots and Cd concentrations in wheat kernels. These findings indicate that the Cd concentration in shoots at an early plant stage can be a suitable indicator of the level of Cd accumulation in kernels. This result is in line with previous results showing that Cd levels at early growth are a useful indicator for Cd contents in grains of genotypes from spring, durum and winter wheat (Greger and Löfstedt 2004; Stolt et al. 2006). Furthermore, Stolt et al. (2006) showed that the variation of Cd accumulation between genotypes remained consistent, regardless of soil type or growing season, suggesting that the results from the present study could be generalised to other growth conditions and that inherent crop properties are the most important for Cd levels in spring wheat.

5 Conclusions

It was hypothesised that differences of Cd uptake and distribution in spring wheat genotypes could result from different abilities to mobilise Cd from soil particles and exclude Cd at the root surface and differences in the exudation of amino acids and organic acids.

The results highlighted that neither the root exudation pattern nor the Cd uptake and distribution were correlated with Cd mobility in the rhizosphere soil, which implies that direct mechanistic links between root exudation and plant Cd uptake are unlikely to occur in wheat. However, it was shown that a higher Cd exclusion from the root leads to a lower Cd uptake and that root exudation composition influenced Cd distribution in the plants, but not Cd uptake. Indeed, the results showed that acetate and succinate exudation influenced shoot Cd concentrations, and that Cd exclusion from the roots was associated with the exudation level of deoxyribonucleosides. Our observations, which were mainly based on correlations, can be used as predictions for further mechanistic investigations to causally understand how root exudates can influence Cd transport, sequestration and exclusion from roots.

By identifying the relationship between the exudation level of some compounds and Cd concentration in different wheat plant parts, this study could help identify plant traits associated with Cd uptake and distribution in wheat and particularly in grains. Our results provide important information for plant breeders working on the development of low Cd accumulation varieties by helping them focus on genes and/or phenotypes involved in Cd uptake and translocation in spring wheat.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The research did not involve humans or animal research.

Competing Interests The authors declare no competing interests.

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