



## Research article

Genetic analysis of cadmium accumulation in lettuce (*Lactuca sativa*)

Walid Zorrig<sup>a,b</sup>, Jean-Yves Cornu<sup>c,\*</sup>, Brigitte Maisonneuve<sup>d</sup>, Aïda Rouached<sup>a</sup>, Catherine Sarrobert<sup>e</sup>, Zaïgham Shahzad<sup>a</sup>, Chedly Abdelly<sup>b</sup>, Jean-Claude Davidian<sup>a</sup>, Pierre Berthomieu<sup>a</sup>

<sup>a</sup> BPMP, Montpellier SupAgro, CNRS, INRA, Université de Montpellier, France

<sup>b</sup> Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, Hammam-Lif 2050, Tunisie

<sup>c</sup> ISPA, Bordeaux Sciences Agro, INRA, 33140, Villenave d'Ornon, France

<sup>d</sup> INRA, UR Génétique et Amélioration des Fruits et Légumes, 1052 Domaine St Maurice, BP 94, 84143, Montfavet Cédex, France

<sup>e</sup> Groupe de Recherches Appliquées en Phytotechnologie, DEVM, CEN Cadarache, 13108, St Paul les Durance, France

## ARTICLE INFO

## Keywords:

Lettuce  
Diversity analysis  
Cadmium accumulation  
Cadmium tolerance  
Genotype

## ABSTRACT

This work characterized mechanisms controlling cadmium (Cd) tolerance and accumulation in lettuce at both the physiological and genetic levels. These traits were evaluated in 18 *Lactuca* accessions representing a large genetic diversity. Cd tolerance and accumulation in roots and shoots as well as Cd translocation from roots to the shoot varied independently, and with a significant range of variation. Analyses of F1 progenies of crosses between cultivars with contrasted phenotypes showed that high tolerance to Cd, low Cd accumulation and low Cd root-shoot translocation were recessive traits. Results of analyses of F2 progenies of different crosses suggest that root Cd concentration and root-shoot Cd translocation were under a complex genetic determinism involving at least two loci. This work thus revealed that limiting both Cd accumulation and Cd root-shoot translocation in lettuce is possible and depends on recessive loci. Differences in the ability to accumulate Cd in roots in the long term could not be linked to differences in short-term <sup>109</sup>Cd uptake into, or efflux from, roots. In contrast, the cultivar with the highest root-shoot Cd translocation was the same in the long term and in the short term, which suggests that this trait relies on processes that are implemented quickly (i.e. in less than three days) after the start of Cd exposure.

## 1. Introduction

Anthropogenic activities such as urban traffic, heating systems, the use of electric batteries or agricultural fertilizers have been increasing both organic and inorganic pollution, including Cd pollution, since the industrial revolution. Inorganic pollutants, in particular heavy metals, cannot be degraded. As a result, their concentrations are continuously increasing in water resources and in the upper horizons of soils. Cadmium is a non-essential, heavy metal with an extremely long biological half-life of more than 20 years. Chronic Cd poisoning can damage the kidneys and bones as well as cause cancer (Godt et al., 2006; IARC, 1993).

Cadmium concentrations are also increasing in cultivated soils, notably due to the use of Cd-containing P fertilizers (Alloway and Steiness, 1999). Crops absorb and accumulate Cd including in their edible parts. As a result, Cd enters the food chain and contaminates human beings. For instance, between 1 and 5% of French durum wheat grains contain more than the European regulatory limit of 0.2 mg kg<sup>-1</sup>

fresh weight fixed for Cd (Mench and Baize, 2004). Several other crops including rice, sunflower, potato and soybean also accumulate significant levels of Cd (Grant et al., 2008). Lettuce (*Lactuca sativa*) accumulates high concentrations of Cd in its leaves (Costa and Morel, 1994; Mench and Baize, 2004; Zare et al., 2018; Zorrig et al., 2013) so that the leaf Cd concentration in lettuce sometimes surpasses the European regulatory limit fixed for Cd, even in Cd-untamined fields (Baldantoni et al., 2016; Tang et al., 2016). Appropriate agronomic practices can contribute to reduce the uptake and accumulation of Cd in plant tissues (Rizwan et al., 2017). However, it is also extremely important to breed new commercial varieties that accumulate less Cd in their edible parts (Grant et al., 2008).

Diversity in Cd accumulation has already been reported in several plant species including *Arabidopsis thaliana* (Chao et al., 2012), rice (Huang et al., 2015), durum wheat (Perrier et al., 2016), barley (Wu et al., 2015), *Brassica napus* (Chen et al., 2018), maize (Zhao et al., 2018) or lettuce (Zhang et al., 2013). As a result, new low-Cd varieties of wheat (Clarke et al., 2006) and sunflower (Miller et al., 2006),

\* Corresponding author.

E-mail address: [jean-yves.cornu@inra.fr](mailto:jean-yves.cornu@inra.fr) (J.-Y. Cornu).

<https://doi.org/10.1016/j.plaphy.2019.01.011>

Received 10 September 2018; Received in revised form 8 January 2019; Accepted 9 January 2019

Available online 10 January 2019

0981-9428/ © 2019 Elsevier Masson SAS. All rights reserved.

among other species, have already been bred. In several instances, the main reason for low Cd accumulation in grain or in shoot was a reduction in the root-shoot translocation of Cd. This was particularly clear in rice (Uraguchi et al., 2009), in which the tonoplasmic transporter OsHMA3 is hypothesized to be the genetic factor controlling this trait (Miyadate et al., 2011; Ueno et al., 2010). It was also clear in *A. thaliana* and in maize, in which orthologues of the HMA family controlling Cd root-shoot translocation were found to be responsible for the variations in the shoot Cd concentration (Chao et al., 2012; Zhao et al., 2018). In contrast, in *B. napus* and in poplar, the variation in Cd translocation was not underlying the variation in Cd accumulation observed in the shoot (Chen et al., 2018; Induri et al., 2012). Other transporters such as IRT1, CAX or NRAMP as well as phytochelatin were, in those cases, contributing to the observed variation in shoot Cd.

In contrast with all the above-mentioned species, and although Cd is a real problem in lettuce, very few information is available on the diversity of the Cd response within the lettuce species as well as on the genetic architecture underlying this diversity. Only one report comparing more than four different accessions details the response to Cd in lettuce (Zhang et al., 2013). This work enlightened the existence of a great diversity within the lettuce species, but did not go further to identify mechanisms underlying the observed diversity. In this work, we started to analyze the response of lettuce to Cd through the development of a genetic approach. First, a diversity analysis was performed to characterize Cd tolerance, accumulation and root-shoot translocation in a set of 18 *Lactuca* accessions including 16 lettuce cultivars and 2 wild accessions. Subsequently, genetic and physiological analyses were conducted in order to gain information on the mechanisms that could be responsible for the variation observed for these traits.

## 2. Materials and methods

### 2.1. Plant materials

Sixteen old commercial cultivars of lettuce (*Lactuca sativa*) were used in this work (Table 1). Fenja, Pia, Remus and Roxette are from Rijk Zwaan, Divina from Vilmorin, Cobham Green from Tozer, Delsay and

Kordaat from Sluis & Groot, Oresto from Royal Sluis, GL659, Parris Island Cos and Saladin from USA, Mélina from INRA, Pierre Bénite from France, Ruby from USDA and Red Salad Bowl is an old European cultivar. In addition, two accessions of the wild *Lactuca serriola* species were used (Table 1). CR01 was given to INRA by I. Crute (Well-esbourne, GB) in 1981 and LS239 was collected in the Parisian basin (France) by INRA in 1982. Seeds of all these accessions were provided by B. Maisonneuve from INRA genetic resources (UR GAFL, Montfavet, France). All the accessions are pure lines.

F1 progenies were produced by manual crosses between cultivars of contrasted phenotypes. F1 from Kordaat and Parris Island Cos was studied for cadmium accumulation, F1 from Kordaat and Red Salad Bowl for cadmium translocation and F1 from Roxette and Delsay for cadmium tolerance. Morphological characters such as anthocyanin or leaf shape were used to control that the F1 plants were hybrids and not issued from the female parent by self-pollination. For each of the two F1 hybrids made from Kordaat, a F2 population was obtained by self-pollination. In each experiment performed with that material, F1 hybrids and F2 progenies were compared with the parents.

### 2.2. Growing conditions

The following growing conditions were used for all the analyses performed in this work, including the analysis of the different *Lactuca* accessions, as well as the analysis of F1 and F2 progenies. For germination, the seeds were placed on sterile Whatman paper previously moistened with distilled water for five days at 20 °C in the dark and then with a nutrient solution for five more days. The nutrient solution contained 2.5 mM KNO<sub>3</sub>, 0.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.5 mM MgSO<sub>4</sub>, 0.1 mM Fe(III)NaEDTA, 0.05 mM H<sub>3</sub>BO<sub>3</sub>, 0.05 mM MnSO<sub>4</sub>, 15 μM ZnSO<sub>4</sub>, 3 μM Na<sub>2</sub>MoO<sub>4</sub>, 2.5 μM KI, 0.05 μM CuSO<sub>4</sub> and 0.044 μM CoCl<sub>2</sub>. Ten-day-old plantlets were transferred onto a floating support and grown hydroponically. Eight liters of nutrient solution aerated by air bubbling were used for each set of 24 plantlets. After a further four days, CdCl<sub>2</sub> was added to the culture medium at a final concentration of 15 μM and the plantlets were left for eight more days. In the Cd-free control, no CdCl<sub>2</sub> was added to the nutrient solution. The nutrient

**Table 1**

Complete set of data showing the relative growth of roots and shoot, the concentration of Cd in roots and shoot and the root-shoot Cd translocation measured in 16 lettuce (*Lactuca sativa*) cultivars and two wild lettuce (*L. serriola*) accessions exposed to 15 μM Cd for eight days. The relative shoot (resp. root) growth was determined as the ratio of the shoot (resp. root) dry weight (DW) measured in presence of Cd to the shoot (resp. root) dry weight measured in the Cd-free control. Values are means ± SE of 7 individual plants.

Genotype	Species	Cultigroup	Shoot growth			Root growth			Cd accumulation		Cd translocated
			DW in the control	DW in 15 μM Cd	Relative growth	DW in the control	DW in 15 μM Cd	Relative growth	Shoot Cd	Root Cd	%
			mg	mg	%	mg	mg	%	μg g <sup>-1</sup> DW	μg g <sup>-1</sup> DW	
Divina	<i>L. sativa</i>	butterhead	71 ± 5	41 ± 4	58	15 ± 2	11 ± 1	76	327 ± 21	747 ± 63	60 ± 1
Fenja	<i>L. sativa</i>	butterhead	90 ± 5	43 ± 4	48	21 ± 1	12 ± 1	58	369 ± 27	1001 ± 70	58 ± 1
Cobham Green	<i>L. sativa</i>	butterhead	90 ± 12	36 ± 4	40	20 ± 2	9 ± 1	46	351 ± 33	748 ± 80	65 ± 2
Kordaat	<i>L. sativa</i>	butterhead	83 ± 4	41 ± 4	49	19 ± 1	11 ± 1	61	375 ± 32	1029 ± 134	57 ± 2
Mélina	<i>L. sativa</i>	butterhead	79 ± 6	38 ± 3	48	18 ± 3	11 ± 1	60	361 ± 36	852 ± 85	60 ± 2
Oresto	<i>L. sativa</i>	butterhead	44 ± 4	31 ± 2	70	9 ± 1	8 ± 1	96	369 ± 23	820 ± 22	62 ± 1
Pia	<i>L. sativa</i>	butterhead	60 ± 3	32 ± 3	53	13 ± 1	9 ± 1	72	372 ± 28	805 ± 63	63 ± 1
Parris Island Cos	<i>L. sativa</i>	cos	106 ± 6	54 ± 9	51	20 ± 1	13 ± 2	67	251 ± 28	507 ± 20	67 ± 1
Remus	<i>L. sativa</i>	cos	71 ± 4	37 ± 8	52	13 ± 1	10 ± 1	73	295 ± 44	654 ± 74	62 ± 2
Delsay	<i>L. sativa</i>	batavia	66 ± 3	54 ± 3	82	14 ± 1	18 ± 1	135	366 ± 39	659 ± 75	63 ± 2
Pierre Bénite	<i>L. sativa</i>	batavia	78 ± 9	44 ± 6	57	18 ± 4	13 ± 2	74	373 ± 37	817 ± 82	61 ± 1
GL 659	<i>L. sativa</i>	iceberg	82 ± 8	35 ± 5	43	17 ± 2	8 ± 1	50	325 ± 17	733 ± 40	65 ± 1
Roxette	<i>L. sativa</i>	iceberg	70 ± 5	24 ± 6	34	14 ± 1	6 ± 2	42	267 ± 27	662 ± 82	64 ± 2
Saladin	<i>L. sativa</i>	iceberg	78 ± 7	42 ± 4	54	15 ± 2	9 ± 1	60	300 ± 19	731 ± 44	65 ± 1
Red Salad Bowl	<i>L. sativa</i>	cutting	72 ± 5	35 ± 4	48	15 ± 1	8 ± 1	58	379 ± 42	597 ± 64	72 ± 1
Ruby	<i>L. sativa</i>	cutting	53 ± 4	30 ± 4	57	10 ± 1	7 ± 1	70	273 ± 23	602 ± 35	65 ± 1
LS239	<i>L. serriola</i>		26 ± 4	17 ± 3	65	8 ± 2	6 ± 1	71	422 ± 42	788 ± 91	62 ± 2
CR01	<i>L. serriola</i>		26 ± 4	10 ± 1	40	6 ± 1	3 ± 1	51	542 ± 37	896 ± 33	68 ± 2

medium was changed at four-day intervals throughout the experiment. Growth conditions were as follows all along the plant growth: temperature 20 °C, relative humidity 70%, light-dark cycle 16 h:8 h, with a light intensity of 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . At the end of the experiment, the roots were dipped for 12 s in three separate ice-cold 0.5 mM  $\text{CaCl}_2$  solutions to remove the Cd adsorbed on the surface of the root and the roots were carefully dried between two layers of filter paper.

In all the experiments, plants were analyzed one by one and their roots and shoots were harvested and analyzed separately. After harvest, samples were dried at 80 °C for 48 h. Shoot (resp. root) Cd tolerance was determined as the ratio of the shoot (resp. root) dry weight of at least eight 22-day-old plants grown for the preceding eight days in 15  $\mu\text{M}$   $\text{CdCl}_2$  to the shoot (resp. root) dry weight of at least eight plants grown in the Cd-free control.

### 2.3. Cadmium concentration measurements

The weighed dried root or shoot samples were incubated in 1 N  $\text{H}_2\text{SO}_4$  at 80 °C for 30 min to extract ions. Cadmium concentration in the extracts was determined by flame atomic absorption spectrophotometry (SpectrAA 220, Varian, Australia) and was the mean of three analytical replicates. Root-shoot Cd translocation was calculated as the ratio of the quantity of Cd accumulated in the shoot vs. the total quantity of Cd accumulated in both the roots and the shoot.

### 2.4. Influx, efflux and translocation measurements

Influx, efflux and translocation measurements made with  $^{109}\text{Cd}$  were performed using 3-week-old plants grown in the nutrient solution described above in which  $\text{CdCl}_2$  had been added at the concentration of 15  $\mu\text{M}$  for the last three days. To measure root Cd influx, the plants were bathed individually for 20 min in 50 mL of nutrient solution supplemented with 5 mM MES (pH 5.8) and 15  $\mu\text{M}$   $\text{CdCl}_2$ , radiolabeled with 0.44 MBq  $\text{L}^{-1}$   $^{109}\text{Cd}$  ( $^{109}\text{CdCl}_2$ , PerkinElmer) and aerated by air bubbling. At the end of the exposure period, the roots were dipped in two successive 50 mL ice cold desorption baths composed of both 5 mM  $\text{CaCl}_2$  and 15  $\mu\text{M}$   $\text{CdCl}_2$  for 5 min to remove the radioactivity present in the apoplast (Buckley et al., 2010). The roots and shoots were then separated, wiped, weighed, mineralized in a 1:1 mix of 75% perchloric acid and 70% nitric acid at 60 °C and radioactivity was determined by liquid scintillation counting (TRI-CARB 2100 TR, Packard). Since almost no radioactivity was detected in shoot tissues at the end of the 20-min-long incubation period, the influx of Cd was calculated for each plant from the radioactivity measured in root tissues and from the root dry weight.

To measure the root Cd efflux, the plants were bathed for 3 h in 50 mL of a  $^{109}\text{Cd}$  radiolabeled nutrient solution similar to the one used for the root Cd influx. After exposure, the roots were dipped for 5 min in two successive desorption baths (as above) and then transferred in a 50 mL efflux solution for 1 h at 20 °C. The efflux solution consisted in a nutrient solution supplemented with 5 mM MES (pH 5.8) and 15  $\mu\text{M}$   $\text{CdCl}_2$ . To monitor the efflux of  $^{109}\text{Cd}$  from root tissues over time, 1 mL of efflux solution was collected after 5, 10, 30 and 60 min and counted by liquid scintillation. The roots and leaves of each plant were then separated, wiped, weighed, mineralized and counted as described above for the influx measurements. The root Cd efflux was calculated as the ratio of the total radioactivity measured in the efflux solution to that measured in the roots.

Finally, the ratio of radioactivity detected in the shoot at the end of the 3 h labeling period to the sum of radioactivity detected in root + shoot tissues was calculated to estimate the fraction of absorbed Cd allocated to the shoot.

### 2.5. Statistical analyses

One-way ANOVA was used for parametric or non-parametric

comparison of means. Significant differences between accessions were further analyzed using Tukey's parametric or non-parametric tests. All these tests used an alpha of 0.05 and were performed using ©R 2.6.2 statistical software (R Core Team, 2008). Principal Component Analysis (PCA) was performed using XLSTAT ([www.xlstat.com](http://www.xlstat.com)). All the variables were centered around their means and normalized with a standard deviation of 1.

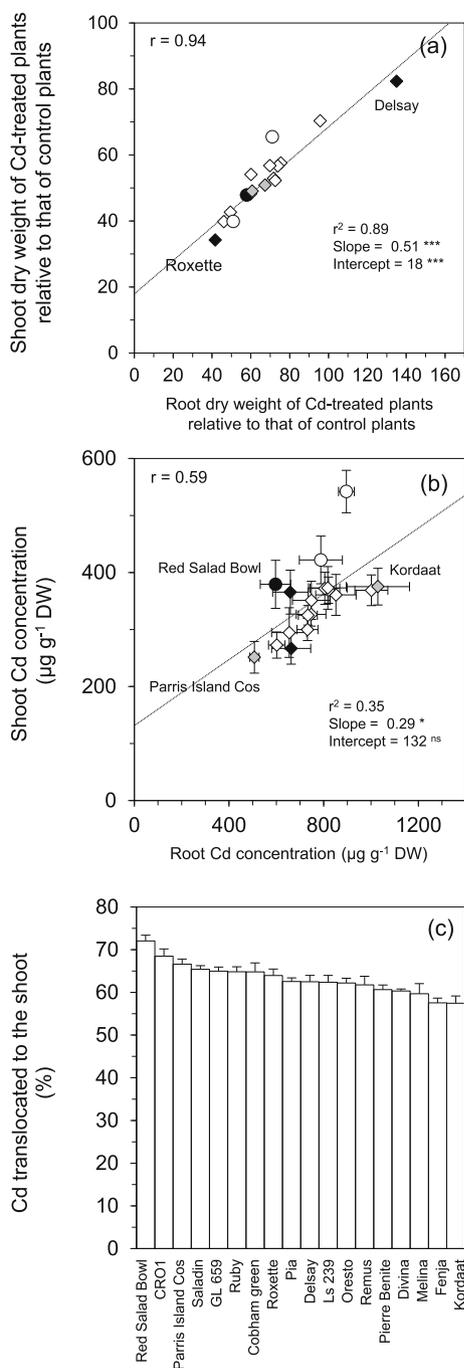
## 3. Results

### 3.1. Analysis of the diversity of cadmium tolerance and cadmium accumulation in lettuce

The responses to Cd of 18 accessions representing the genetic diversity of *Lactuca* were analysed in this study. Sixteen of these accessions corresponded to *L. sativa* old commercial cultivars representing the five cultigroups cos, butterhead, crisphead batavia type, crisphead iceberg type and cutting. The remaining two accessions corresponded to two genotypes of the wild lettuce *L. serriola* (Table 1). The plants were grown for 14 days in normal nutrient solution then for eight additional days in a normal nutrient solution supplemented with  $\text{CdCl}_2$  at a final concentration of 15  $\mu\text{M}$ . Controls were grown with no addition of Cd over the final eight days.

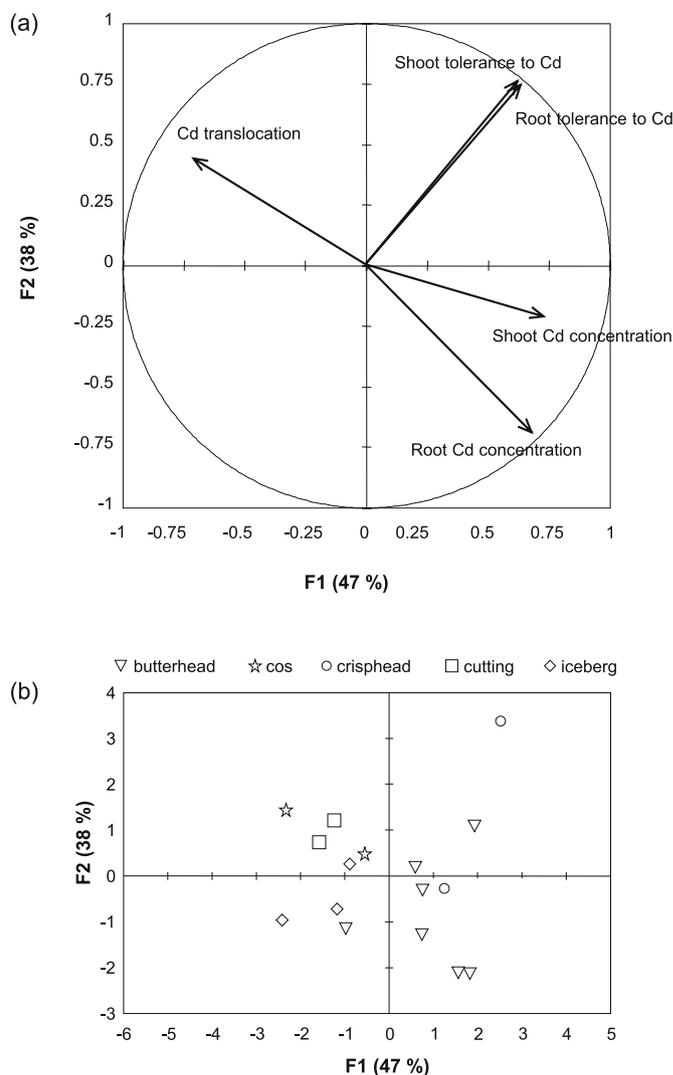
Cadmium tolerance was estimated by comparing biomass production between Cd-treated plants and control plants. The Cd treatment had a detrimental effect on both root and shoot biomasses, the latter being more strongly affected than the former (Fig. 1a). High variability was observed among the 16 *L. sativa* cultivars: shoot and root dry weights of Cd treated plants varied from respectively 34%–82% and from 42% to 135% compared to controls (Fig. 1a; Table 1). A strong correlation was observed between the effects of Cd on the root and shoot dry weights ( $r = 0.94$ ;  $p < 10^{-8}$ ) (Fig. 1a). No clear relationship was found between the observed level of Cd tolerance and the cultigroup to which the *L. sativa* accessions belonged. Considering Cd tolerance, the two accessions representing the wild *L. serriola* did not differ markedly from that of the lettuce cultivars. However, they grew very slowly as compared to the *L. sativa* cultivars, including in the Cd-free control.

Cadmium concentrations were on average twice higher in roots than in shoots (Fig. 1b; Table 1). Root Cd concentrations ranged from 500 to 1000  $\mu\text{g g}^{-1}$  DW while the Cd concentration in the shoot ranged from 250 to 540  $\mu\text{g g}^{-1}$  DW. These values may appear very high compared to those observed in lettuce plants grown on soil, even on Cd-polluted soils. However, they are in full agreement with the values we obtained in previous experiments (Zorrig et al., 2010, 2013) and with the values reported by other groups when they used high concentrations of Cd in the nutrient solution (for recent reports see Matraszek et al., 2016; Zare et al., 2018). There was a significant linear relationship between Cd concentrations in the roots and in the shoot ( $r = 0.59$ ;  $p = 0.01$ ; Fig. 1b). However, the two wild *L. serriola* accessions differed markedly from this trend. While their root Cd concentration was close to that of the *L. sativa* cultivars, their shoot Cd concentration was noticeably higher. Indeed, the concentration of Cd did not exceed 380  $\mu\text{g Cd g}^{-1}$  DW in the shoots of the cultivars, whereas it reached 420 and 540  $\mu\text{g Cd g}^{-1}$  DW in the shoots of the *L. serriola* accessions. The two *L. sativa* cultivars Delsay and Red Salad Bowl also differed slightly from the general trend (Fig. 1b; Table 1). Compared to the other cultivars, the concentrations of Cd in their shoot were rather high (370–380  $\mu\text{g Cd g}^{-1}$  DW) whereas the concentrations of Cd in their roots were among the lowest (600–650  $\mu\text{g Cd g}^{-1}$  DW). Removing data concerning the wild lettuce accessions and Delsay and Red Salad Bowl improved the quality of the linear relationship linking root and shoot Cd concentrations among the remaining 14 lettuce accessions ( $r = 0.87$ ;  $p = 6 \cdot 10^{-5}$ ). This observation suggests that, considering the set of lettuce accessions we considered, the main factor underlying the variation in Cd accumulation in lettuce shoot is the ability of the plant to take up Cd



**Fig. 1.** Diversity analysis of Cd tolerance (a), Cd accumulation (b) and Cd translocation from roots to shoots (c) in 18 *Lactuca* accessions. Each point corresponds to one accession. White circles (○) represent the two *L. serriola* accessions, black diamonds (◆) represent the two accessions showing extreme tolerance (Delsay and Roxette), grey diamonds (◊) represent the two accessions showing extreme accumulation (Kordaat and Parris Island Cos) and black circles (●) represent the accession showing the highest root-shoot Cd translocation (Red Salad Bowl). Values are means ± one standard error of seven individuals. In (a) and (b), the dashed line represents the best linear fit obtained between the two variables (n = 18).

from the soil. Only two lettuce cultivars and one wild accession were to some extent departing from this trend. Interestingly, there was a strong relationship between root or shoot Cd concentrations and the cultigroup to which the accessions belonged. For instance, the eight cultivars with the highest root Cd concentrations included all seven butterhead accessions in the panel. The other cultigroups were represented



**Fig. 2.** Principal component analysis of Cd tolerance, Cd accumulation and Cd translocation from roots to shoots in 16 *L. sativa* accessions. Five variables (a) and the 16 different accessions (b) are projected onto the F1–F2 principal factorial plane that explains 85% of the variation. The cultigroup to which each accession belongs was added as a qualitative supplementary variable.

by only two or three accessions, which makes them difficult to compare. However, the cos and cutting accessions were characterized by the lowest root Cd concentrations.

The root-shoot translocation of Cd also varied among the 18 accessions (Fig. 1c, Table 1). Red Salad Bowl allocated the highest proportion (72%) of the Cd taken up by the roots to the shoot, and Kordaat and Fenja the lowest (57%). The two wild accessions did not display any specific phenotype for this trait. In contrast, the performances of the cultivars were cultigroup dependent. On average, cutting and iceberg accessions allocated a higher proportion of Cd to the shoot than butterhead and cos accessions.

The trait-by-trait analyses were completed by a PCA (Fig. 2; Tables 2 and 3). The PCA was restricted to *L. sativa* cultivars as the *L. serriola* accessions had specific and significantly different growth and ability to accumulate Cd in the shoot. Indeed, including the *L. serriola* data into the PCA analysis biased the results towards enlightening and emphasizing the differences between *L. sativa* and *L. serriola*, which did not correspond to our main interest, which was to analyse the *L. sativa* intra-species variation in response to Cd. A strong positive correlation ( $r = 0.97$ ;  $p < 0.05$ ) was observed between Cd tolerance in roots and shoots, confirming previous analyses of these traits (Fig. 1a). Root and

**Table 2**

Pearson's correlation matrix of Cd tolerance, Cd concentration in roots and shoot and root-shoot Cd translocation, in the 16 *Lactuca sativa* accessions exposed to 15  $\mu\text{M}$  CdCl<sub>2</sub>.

Variables	Relative shoot growth	Relative root growth	Shoot Cd concentration	Root Cd concentration	Cd translocation
Relative shoot growth	1	<b>0.967</b>	0.270	−0.063	−0.146
Relative root growth		1	0.256	−0.108	−0.159
Shoot Cd concentration			1	<b>0.649</b>	−0.307
Root Cd concentration				1	− <b>0.787</b>
Cd translocation					1

Variables were centred around their means and normalized with a standard deviation of 1, n = 7.

Values in bold represent significant correlations at the 0.05 level.

**Table 3**

Pearson's correlation matrix of the relationship between the traits of Cd tolerance, Cd accumulation and root-to-shoot Cd partitioning, and the cultigroups to which the accessions belong.

Variables	Butterhead	Cos	Batavia	Cutting	Iceberg
Relative shoot growth	−0.037	−0.043	<b>0.579</b>	−0.009	−0.399
Relative root growth	−0.068	0.027	<b>0.637</b>	−0.080	−0.409
Shoot Cd concentration	<b>0.538</b>	− <b>0.546</b>	0.310	−0.076	−0.420
Root Cd concentration	<b>0.714</b>	−0.466	−0.027	−0.413	−0.139
AR Cd ratio	− <b>0.578</b>	0.134	−0.151	<b>0.586</b>	0.252

Variables were centred around their means and normalized with a standard deviation of 1, n = 7.

Values in bold represent significant correlations at the 0.05 level.

shoot Cd concentrations were also positively correlated with each other ( $r = 0.65$ ;  $p < 0.05$ ), but only the former showed a significant (negative) correlation ( $r = -0.79$ ;  $p < 0.05$ ; Table 2) with root-shoot translocation of Cd. Interestingly, no significant correlation was observed between Cd tolerance on the one hand, and the concentration of Cd in the roots and shoot or the root-shoot Cd translocation on the other hand (Table 2). This is also in agreement with the results of the trait-by-trait analyses. Indeed, cultivars with extreme phenotypes for Cd accumulation or root-shoot Cd translocation had similar performances for Cd tolerance (Fig. 1a). Conversely, cultivars with extreme phenotypes for Cd tolerance had similar performances for Cd accumulation and root-shoot Cd translocation (Fig. 1b).

The number of cultivars representing each cultigroup was low (between 2 and 7) in all cases, but marked differences between cultigroups were nevertheless observed (Fig. 2b; Table 3). Batavia cultivars were characterized by high Cd tolerance. Butterhead cultivars were characterized by high root and shoot Cd concentrations in contrast to cos cultivars with low shoot Cd concentrations. Cutting cultivars were characterized by a high root-shoot Cd translocation in contrast to butterhead cultivars.

### 3.2. Genetic characterization of the 'cadmium tolerance' trait

Genetic determinism behind the Cd tolerance trait was analyzed through the phenotypic characterization of F1 progenies from crosses between the Delsay and Roxette cultivars, which showed extreme, contrasted phenotypes for this trait. F1 plants from reciprocal crosses between the two parental lines displayed similar Cd tolerance levels to the Roxette parent plants (Fig. 3a). Since Roxette plants displayed the lowest values for this character, the allele(s) responsible for low Cd tolerance showed complete dominance over the allele(s) responsible for high Cd tolerance. This character could not be checked in the F2 progeny. Indeed, Cd tolerance is evaluated in this study as the ratio of dry weight of Cd-treated plants to that of control plants. In a F2 progeny, every plant is unique – i.e. genetically different from its sisters – and it is impossible to evaluate the tolerance of every unique plant to Cd.

### 3.3. Genetic characterization of the 'cadmium accumulation' trait

The genetic determinism of Cd accumulation in roots and shoots was characterized through analyses of F1 and F2 progenies from crosses between the Parris Island Cos and Kordaat cultivars, which showed extreme, contrasted concentrations of Cd in their roots and shoots. The root and shoot Cd concentrations in F1 plants from reciprocal crosses between the two parental lines were similar to those of their Kordaat parent plants (Fig. 3b). Since Kordaat plants had the highest root and shoot Cd concentrations, analysis of the F1 progeny provided evidence that the allele(s) responsible for high Cd concentration is(are) dominant. Cadmium concentration was further analyzed in the roots of 165 F2 plants from the self-progeny of one F1 plant (Fig. 4a). Continuous variation and no significant transgression beyond the phenotypic distributions of the parental cultivars were observed. It was not possible to fit the observed distribution to a bimodal distribution for which the two modes would be centered on the average values of the parent lines ( $p > 0.05$ ). This suggests that the root Cd concentration is not governed by a single locus.

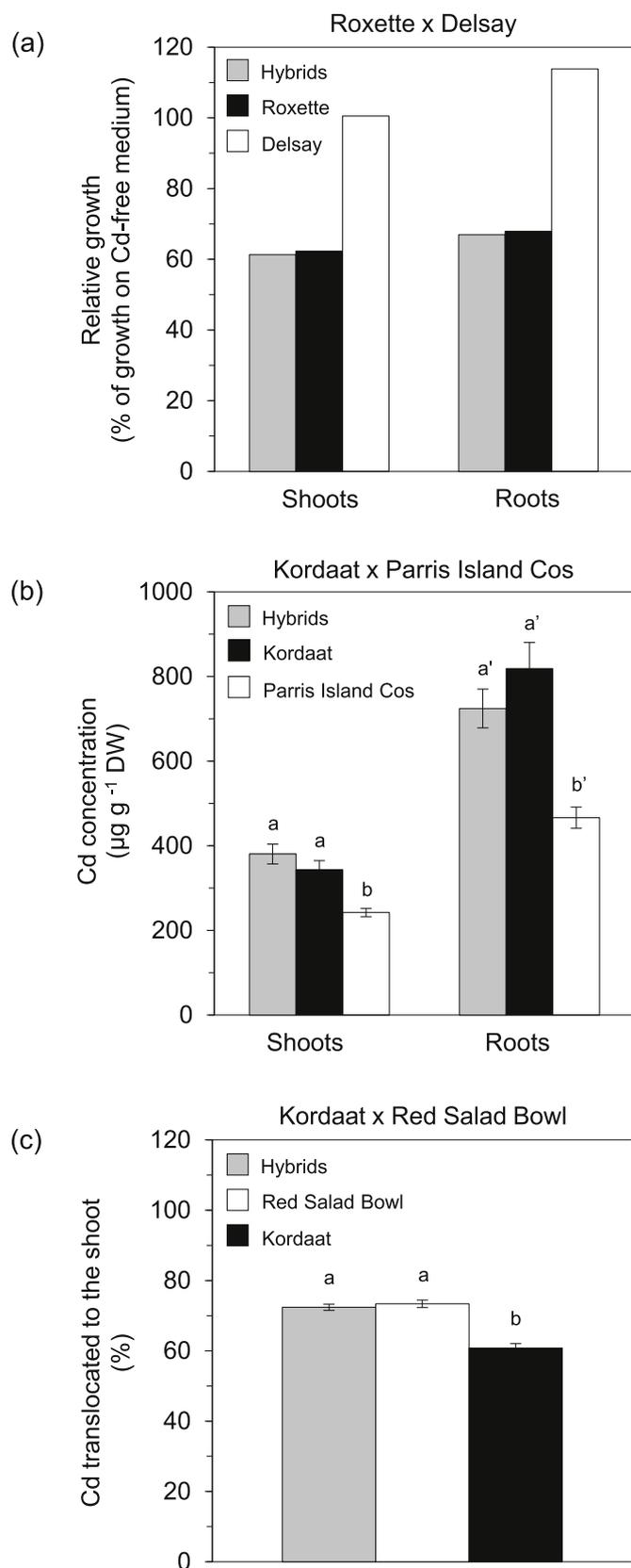
### 3.4. Genetic characterization of the 'root-shoot cadmium translocation' trait

The genetic determinism of the root-shoot translocation of Cd was characterized by analyzing F1 and F2 progenies from crosses between the Red Salad Bowl and Kordaat cultivars, which showed extreme and contrasted phenotypes for this trait. The levels of Cd translocation in F1 plants from reciprocal crosses between the two parental lines were similar to those in the Red Salad Bowl parent plants (Fig. 3c). Since Red Salad Bowl plants had the highest level of root-shoot Cd translocation, the allele(s) responsible for this character showed complete dominance. Root-shoot Cd translocation was further analyzed in 150 F2 plants from the self-progeny of one F1 plant (Fig. 4b). No significant transgression beyond the phenotypic distributions of parental accessions was observed. It was not possible to fit the observed distribution to a bimodal distribution for which the two modes would be centered on the average translocation values observed in the parent lines ( $p > 0.05$ ). This again suggests that root-shoot translocation of Cd is not governed by a single locus.

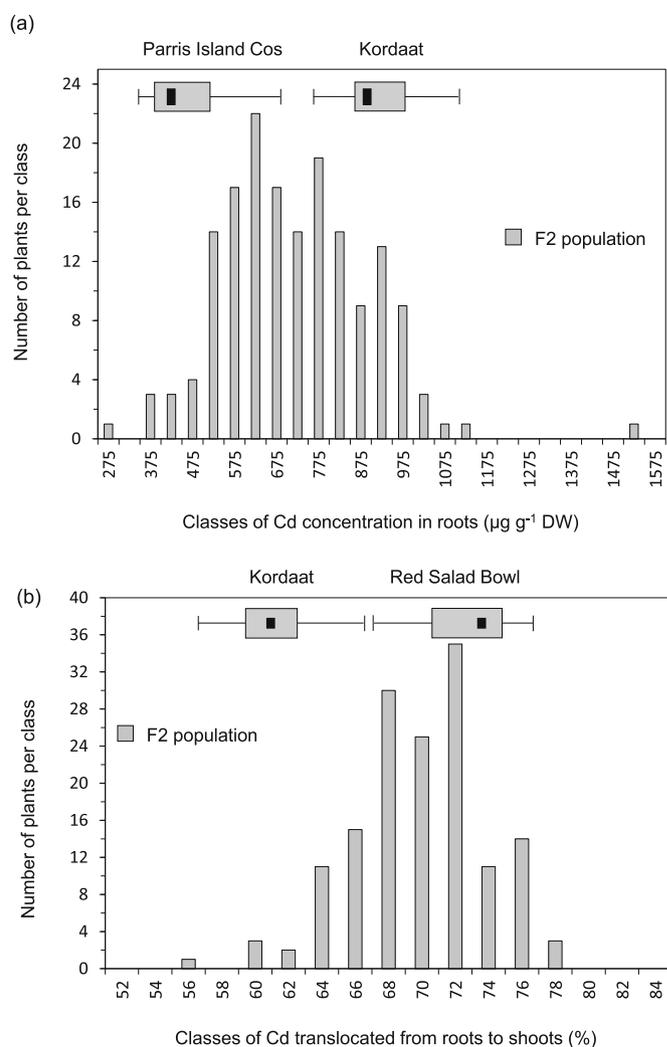
### 3.5. Influx, efflux and root-shoot Cd translocation assessed by <sup>109</sup>Cd measurements

To identify the physiological cause(s) of the diversity in Cd accumulation in roots, Cd influx in roots, Cd efflux from roots and root-shoot Cd translocation were analyzed using short-term <sup>109</sup>Cd labeling. The three *L. sativa* cultivars with extreme contrasted phenotypes for these traits (Red Salad Bowl, Parris Island Cos and Kordaat) were used for the analyses (Fig. 5).

In each of the three lettuce cultivars, the kinetics of <sup>109</sup>Cd influx in roots was linear in the first 20 min (data not shown). We consequently measured <sup>109</sup>Cd influx after the roots were exposed to radiolabeled Cd for 20 min. Root Cd influx ranged from 0.86 to 1.1 10<sup>−6</sup>  $\mu\text{mole Cd mg}^{-1}$  root DW s<sup>−1</sup> (Fig. 5a), which is in line with the usual range of

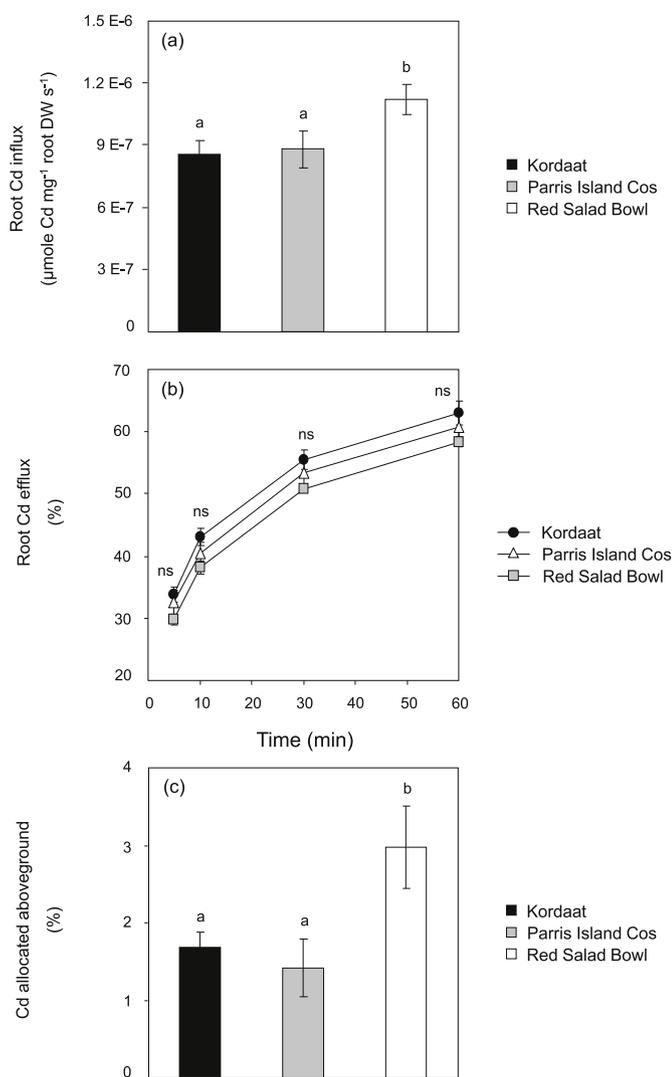


**Fig. 3.** Phenotypic analysis of F1 progenies from crosses between *L. sativa* accessions displaying extreme contrasted performances for Cd tolerance (a), Cd accumulation (b) and root-shoot Cd translocation (c). Values are means  $\pm$  one standard error of eight individuals. Mean values with different letters are significantly different ( $p < 0.05$ ) according to Tukey's test.



**Fig. 4.** Phenotypic analysis of F2 progenies from crosses between *L. sativa* accessions displaying extreme performances for shoot Cd content (a) or root-shoot Cd translocation (b). In panel a, plants showing shoot Cd concentrations ranging in a same 50 ppm range were grouped in a single class and the average concentration of the class is reported on the X-axis. In panel b, plants showing root-shoot Cd translocation ranging in a same 2% range were grouped in a single class and the average percentage of the class is reported on the X-axis. The distributions of the plants representing the parental lines are indicated in box plots. In (a):  $n = 10$  for Kordaat,  $n = 8$  for Parris Island Cos and  $n = 165$  for the F2 population from the Kordaat x Parris Island Cos cross. In (b):  $n = 13$  for Kordaat,  $n = 16$  for Parris Island Cos and  $n = 150$  for the F2 population from the Kordaat x Red Salad Bowl cross.

root Cd influx reported for crops exposed to similar levels of Cd (e.g. Cataldo et al., 1983). Cd influx in roots did not vary significantly ( $p > 0.05$ ) between the Parris Island Cos and Kordaat cultivars but was on average 1.3 times higher ( $p < 0.05$ ) in Red Salad Bowl (Fig. 5a). Measurements of  $^{109}\text{Cd}$  efflux from roots of plants previously grown for 3 h in the presence of  $15 \mu\text{M CdCl}_2$  revealed a comparable decline in Cd efflux over time in all three cultivars ( $p > 0.05$ ; Fig. 5b). In our experimental conditions, for all the three cultivars, around 60% of the Cd assumed to be internalized (Buckley et al., 2010) had moved out of the root cell after 1 h. The distribution of Cd between roots and shoots was analyzed at the end of the 3-h labeling period by measuring the radioactivity in shoot and root tissues (Fig. 5c). Whatever the cultivar, less than 3% of the Cd taken up by the roots was allocated to the shoot during that time period. Nevertheless, plants from the Red Salad Bowl cultivar translocated from 1.5 to 2 times more Cd to the shoot than plants from the Parris Island Cos and Kordaat cultivars ( $p < 0.05$ ).



**Fig. 5.** Root Cd influx (a), efflux of Cd over time expressed as a percentage of the radioactivity in root tissues (b) and Cd allocated aboveground expressed as a percentage of the radioactivity taken up by the plant roots (c), in lettuce accessions displaying contrasted phenotypes of Cd accumulation and translocation. All the results are relative to  $^{109}\text{Cd}$  labeling measurements. Error bars are mean standard errors ( $n = 18$  for root Cd influx,  $n = 12$  for root Cd efflux,  $n = 12$  for Cd allocated aboveground). Mean values with different letters are significantly different ( $p < 0.05$ ) according to Tukey's test and ns stands for non-significant difference.

Taken together, these results suggest that Cd turnover between the medium and lettuce roots was rather high in the three lettuce cultivars considered. No significant difference was observed in Cd uptake, Cd efflux or in the translocation of Cd from the roots to the shoot between Parris Island Cos and Kordaat (Fig. 5), even though these two cultivars had contrasted concentrations of Cd in their roots and shoots after long-term exposure (Fig. 1). Interestingly, the level of root-shoot translocation of Cd by Red Salad Bowl was significantly higher than that of the other cultivars in both short-term (Fig. 5c) and long-term (Fig. 1c) experiments. This was accompanied by a higher root Cd influx (Fig. 5a).

#### 4. Discussion

In agreement with previous studies (Costa and Morel, 1994; Florijn et al., 1991; Zhang et al., 2013), this study shows that lettuce displays significant intra-specific variability for Cd tolerance, Cd accumulation and root-shoot Cd translocation. Interestingly, the three traits appear to

be primarily genetically independent. The only significant correlation was between root Cd concentration and root-shoot Cd translocation. This conclusion may seem surprising as the vast majority of the work published on plant responses to Cd, including on lettuce (Zorrig et al., 2010), report that changes in the transport or accumulation of Cd within plant cells or in the whole plant result in changes in tolerance to Cd. However, it has also been reported, for instance in rice (Xue et al., 2009), wheat (Ci et al., 2011) or in *Averrhoa carambola* (Dai et al., 2011), that Cd tolerance and Cd accumulation could be genetically independent. In order to explain the apparent discrepancy between the different reports, we hypothesize that the number of accessions from which the conclusions were drawn is critical. The greater the number of accessions, the weaker the correlation between Cd tolerance and Cd accumulation. Indeed, alterations of Cd transport and accumulation induced in one particular accession by means of genetic engineering usually result in changes in tolerance to Cd. However, in such a situation, there is no change in the intrinsic sensitivity of the accession, as intrinsic sensitivity is linked to the cellular targets of Cd, the intracellular compartmentalization of Cd, or more generally, the processes that make Cd phytotoxic. In contrast, when different accessions are examined, it is likely they will display variations in their intrinsic sensitivity to Cd at the cell level in addition to displaying differences in their ability to accumulate Cd.

Another interesting result of our analyses is that between-accession variation in leaf Cd concentration was not correlated with variation in root-shoot Cd translocation but rather with variations in the concentration of Cd in the roots. In particular, the concentrations of Cd in the shoot of the two lines showing extreme contrasted performances with regard to Cd translocation were very similar. This result might seem surprising since in *A. thaliana*, *A. halleri*, tobacco and rice, the main factors controlling the accumulation of Cd in the shoot were shown to be transporters from the Zn/Cd  $P_{1B}$ -ATPase (*HMA*) family that control the root-shoot transport of these elements (Hanikenne et al., 2008; Hayes et al., 2013; Miyadate et al., 2011; Satoh-Nagasawa et al., 2012; Ueno et al., 2010; Verret et al., 2004; Wong and Cobbett, 2009). One possible explanation is that all the *L. sativa* cultivars studied here show little, if any, polymorphism at the *HMA* loci. Actually, we observed that most of these cultivars displayed very similar root-shoot Cd translocation. However, it would be of primary interest to analyze in a following study what could be the respective contributions of the different copies of the Cd/Zn  $P_{1B}$ -ATPase present in lettuce (see below) to the Cd concentration in shoot as well as to the root-shoot Cd translocation in the different accessions. One important result of our study is that variation in leaf Cd concentration was primarily correlated with variation in root Cd concentration. This suggests that the net uptake of Cd by roots would be the main factor controlling its accumulation in lettuce shoots at  $15 \mu\text{M}$  Cd. Provided that this conclusion remains true at lower Cd exposure and at more advanced growth stages, this implies that a special attention should be paid to mechanisms and genes underlying Cd uptake in order to limit the accumulation of Cd in lettuce shoot in agricultural context.

To identify the functional trait underlying the between-cultivar variation in the net uptake of Cd, we measured Cd influx and efflux after short-term exposure to  $^{109}\text{Cd}$ -labeled solutions. The cultivars used for these experiments were Kordaat and Parris Island Cos, because of their contrasted ability to accumulate Cd in their roots and shoot on a long-term basis. Surprisingly, the influx and efflux experiments revealed no difference between the two cultivars. This is even more surprising that the plants used for these short-term experiments were acclimated to  $15 \mu\text{M}$  Cd for the last three days before being exposed to  $^{109}\text{Cd}$ , which is likely more than the induction time of the mechanisms involved in Cd homeostasis. This apparent discrepancy between the results of the short-term and long-term experiments suggests a role for "long-term" processes in restricting the movement of Cd from the nutrient solution to the root symplast. Among other regulatory mechanisms, root exudation of organic compounds (Chiang et al., 2006) is a

way to limit the Cd influx into the root symplast, the efficiency of which may differ between cultivars. We hypothesize that this root response to Cd could explain the differences in Cd accumulation observed, at least between Kordaat and Parris Island Cos, in the long-term experiment, but that its impact on the Cd influx could not be emphasized by the protocol of our short-term experiment. Indeed, the Cd influx was measured over 20 min in a fresh nutrient solution, therefore free of organic exudates.

From a genetic point of view, it would be very exciting to identify the genes underlying the observed variability in Cd tolerance, Cd accumulation and root-shoot Cd translocation. In fact, this work is already underway, thanks to the recent release of the sequence of the lettuce genome (Reyes-Chin-Wo et al., 2017). This genome appears to be very large (~2.7 Gbp) and complex. In particular, one fourth of it corresponds to a recent triplication. Consequently, identifying the genes underlying our traits of interest is a complex task. For instance, Cd/Zn ATPases from the HMA gene family appear to be good candidates for the control of root-shoot translocation of Cd (Verbruggen et al., 2009). However, at least two Zn/Cd ATPases had already been identified in lettuce (Zorrig et al., 2011) and the recently released genome sequence suggests that there may be one or two additional paralogs. However, we already discovered in the present work that high tolerance to Cd, low Cd accumulation in plants and low translocation of Cd from the roots to the shoot are recessive traits. This suggests that improvements in these traits will benefit from the identification and use of loss-of-function alleles.

## Contributions

PB and JCD designed experiments. WZ did most of the experiments and analyzed data. BM, AR and CS performed genetic crosses and analyzed recombinant plants. JYC conducted <sup>109</sup>Cd labeling experiments. PB, WZ and ZS wrote the manuscript. BM, AR, CS, JCD, JYC and CA revised the manuscript for important intellectual content.

## Acknowledgements

W. Zorrig was supported by a scholarship from the Tunisian Ministry of Higher Education, Scientific Research and Technology (LR15CBBC02) then by a scholarship from the Agence Universitaire de la Francophonie (AUF). A. Rouached was supported by the Agropolis Foundation grant no. 07010. Z. Shahzad was supported by a scholarship from the Higher Education Commission, Pakistan. We thank A. Adiveze, H. Afonso, C. Baracco, H. Baudot, F. Bourgeois, C. Dasen, X. Dumont, J. Garcia, S. Gélín, F. Lecocq, V. Papy, V. Rafin, G. Ruiz and C. Zicler, for technical and administrative support.

## References

Alloway, B.J., Steinnes, E., 1999. Anthropogenic additions of cadmium to soils. In: McLaughlin, M.J., Singh, B.R. (Eds.), *Cadmium in Soils and Plants*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 97–123.

Baldantoni, D., Morra, L., Zaccardelli, M., Alfani, A., 2016. Cadmium accumulation in leaves of leafy vegetables. *Ecotoxicol. Environ. Saf.* 123, 89–94.

Buckley, W.T., Buckley, K.E., Huang, J.J., 2010. Root cadmium desorption methods and their evaluation with compartmental modeling. *New Phytol.* 188, 280–290.

Cataldo, D.A., Garland, T.R., Wildung, R.E., 1983. Cadmium uptake kinetics in intact soybean plants. *Plant Physiol.* 73, 844–848.

Chao, D.-Y., Silva, A., Baxter, I., Huang, Y.S., Nordborg, M., Danku, J., Lahner, B., Yakubova, E., Salt, D.E., 2012. Genome-Wide association studies identify heavy metal ATPase3 as the primary determinant of natural variation in leaf cadmium in *Arabidopsis thaliana*. *PLoS Genet.* 8, e1002923.

Chen, L., Wan, H., Qian, J., Guo, J., Sun, C., Wen, J., Yi, B., Ma, C., Tu, J., Song, L., Fu, T., Shen, J., 2018. Genome-Wide association study of cadmium accumulation at the seedling stage in rapeseed (*Brassica napus* L.). *Front. Plant Sci.* 9, 375.

Chiang, P.N., Wang, M.K., Chiu, C.Y., Chou, S.Y., 2006. Effects of cadmium amendments on low-molecular-weight organic acid exudates in rhizosphere soils of tobacco and sunflower. *Environ. Toxicol.* 21, 479–488.

Ci, D., Jiang, D., Dai, T., Jing, Q., Cao, W., 2011. Variation in cadmium tolerance and accumulation and their relationship in wheat recombinant inbred lines at seedling stage. *Biol. Trace Elem. Res.* 142, 807–818.

Clarke, J.M., McCaig, T.N., DePauw, R.M., Knox, R.E., Clarke, F.R., Fernandez, M.R., 2006. Registration of 'Strongfield' durum wheat. *Crop Sci.* 46, 2306–2307.

Costa, G., Morel, J.L., 1994. Efficiency of H<sup>+</sup>-ATPase activity on cadmium uptake by four cultivars of lettuce. *J. Plant Nutr.* 17, 627–637.

Dai, Z.Y., Shu, W.S., Liao, B., Wan, C.Y., Li, J.T., 2011. Intraspecific variation in cadmium tolerance and accumulation of a high-biomass tropical tree *Averrhoa carambola* L.: implication for phytoextraction. *J. Environ. Monit.* 13, 1723–1729.

Florijn, P.J., Nelemans, J.A., Van Beusichem, M.L., 1991. Cadmium uptake by lettuce varieties. *Neth. J. Agric. Sci.* 39, 103–114.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., Gronenberg, D., 2006. The toxicity of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol.* 1, 22–27.

Grant, C.A., Clarke, J.M., Duguid, S., Chaney, R.L., 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. *Sci. Total Environ.* 390, 301–310.

Hanikenne, M., Talke, I.N., Haydon, M.J., Lanz, C., Nolte, A., Motte, P., Kroymann, J., Weigel, D., Kramer, U., 2008. Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of *HMA4*. *Nature* 453, 391–395.

Hayes, A., Kudithipudi, C., Van der Hoeven, R., 2013. Transgenic plants modified for reduced cadmium transport, derivative products, and related methods. U.S. Patent No. 8 (383), 889 26 Feb. 2013.

Huang, Y., Sun, C., Min, J., Chen, Y., Tong, C., Bao, J., 2015. Association mapping of quantitative trait loci for mineral element contents in whole grain rice (*Oryza sativa* L.). *J. Agric. Food Chem.* 63, 10885–10892.

IARC, 1993. Evaluation of Carcinogenic Risks to Humans: beryllium, cadmium, mercury and exposures in the glass manufacturing industry. IARC Monographs 58 119–237.

Induri, B.R., Ellis, D.R., Slavov, G.T., Yin, T., Zhang, X., Muchero, W., Tuskan, G.A., DiFazio, S.P., 2012. Identification of quantitative trait loci and candidate genes for cadmium tolerance in Populus. *Tree Physiol.* 32, 626–638.

Matraszek, R., Hawrylak-Nowak, B., Chwili, S., Chwili, M., 2016. Macroelemental composition of cadmium stressed lettuce plants grown under conditions of intensive sulphur nutrition. *J. Environ. Manag.* 180, 24e34.

Mench, M., Baize, D., 2004. Contamination des sols et de nos aliments d'origine végétale par les éléments en trace, mesures pour réduire l'exposition. *Courrier de l'Environnement de l'INRA*, vol. 52. pp. 31–54.

Miller, J.F., Green, C.E., Li, Y.M., Chaney, R.L., 2006. Registration of three low cadmium (HA 448, HA 449, and RHA 450) confection sunflower genetic stocks. *Crop Sci.* 46, 489–490.

Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., Watanabe, A., Fujimura, T., Akagi, H., 2011. OsHMA3, a P<sub>1B</sub>-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol.* 189, 190–199.

Perrier, F., Yan, B., Candaudap, F., Pokrovsky, O.S., Gourdain, E., Meleard, B., Bussiere, S., Coriou, C., Robert, T., Nguyen, C., Cornu, J.Y., 2016. Variability in grain cadmium concentration among durum wheat cultivars: impact of aboveground biomass partitioning. *Plant Soil* 404, 307–320.

R Core Team, 2008. R: a Language and Environment for Statistical Computing. R foundation for statistical computing, Vienna, Austria. <https://www.R-project.org/>.

Reyes-Chin-Wo, S., Wang, Z., Yang, X., Kozik, A., Arikait, S., Song, C., Xia, L., Froenicke, L., Lavelle, D.O., Truco, M.J., Xia, R., Zhu, S., Xu, C., Xu, H., Xu, X., Cox, K., Korf, I., Meyers, B.C., Michelmore, R.W., 2017. Genome assembly with *in vitro* proximity ligation data and whole-genome triplication in lettuce. *Nat. Commun.* 8, 14953.

Rizwan, M., Ali, S., Adrees, M., Ibrahim, M., Tsang, D.C.W., Zia-ur-Rehman, M., Zahir, Z.A., Rinklebe, J., Tack, F.M.G., Ok, Y.S., 2017. A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere* 182, 90–105.

Satoh-Nagasawa, N., Mori, M., Nakazawa, N., Kawamoto, T., Nagato, Y., Sakurai, K., Takahashi, H., Watanabe, A., Akagi, H., 2012. Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. *Plant Cell Physiol.* 53, 213–224.

Tang, X., Pang, Y., Ji, P., Gao, P., Nguyen, T.H., Tong, Y., 2016. Cadmium uptake in above-ground parts of lettuce (*Lactuca sativa* L.). *Ecotoxicol. Environ. Saf.* 125, 102–106.

Ueno, D., Yamajia, N., Konob, I., Huang, C.F., Andob, T., Yanoc, M., Maa, J.F., 2010. Gene limiting cadmium accumulation in rice. *Proc. Natl. Acad. Sci. U.S.A.* 107, 16500–16505.

Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arai, T., Ishikawa, S., 2009. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J. Exp. Bot.* 60, 2677–2688.

Verbruggen, N., Hermans, C., Schat, H., 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr. Opin. Plant Biol.* 12, 364–372.

Verret, F., Gravot, A., Auroy, P., Leonhardt, N., David, P., Nussaume, L., Vavasseur, A., Richaud, P., 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. *FEBS Lett.* 576, 306–312.

Wong, C.K.E., Cobbett, C.S., 2009. HMA P-type ATPases are the major mechanism for root-to-shoot Cd translocation in *Arabidopsis thaliana*. *New Phytol.* 181, 71–78.

Wu, D., Sato, K., Ma, J.F., 2015. Genome-wide association mapping of cadmium accumulation in different organs of barley. *New Phytol.* 208, 817–829.

Xue, D., Chen, M., Zhang, G., 2009. Mapping of QTLs associated with cadmium tolerance and accumulation during seedling stage in rice (*Oryza sativa* L.). *Euphytica* 165, 587–596.

Zare, A.A., Khoshgoftarmansh, A.H., Malakouti, M.J., Bahrami, H.A., Chaney, R.L., 2018. Root uptake and shoot accumulation of cadmium by lettuce at various Cd:Zn ratios in nutrient solution. *Ecotoxicol. Environ. Saf.* 148, 441–446.

Zhang, K., Yuan, J., Kong, W., Yang, Z., 2013. Genotype variations in cadmium and lead accumulations of leafy lettuce (*Lactuca sativa* L.) and screening for pollution-safe cultivars for food safety. *Environ. Sci. Process Impacts* 15, 1245–1255.

- Zhao, X., Luo, L., Cao, Y., Liu, Y., Li, Y., Wu, W., Lan, Y., Jiang, Y., Gao, S., Zhang, Z., Shen, Y., Pan, G., Lin, H., 2018. Genome-wide association analysis and QTL mapping reveal the genetic control of cadmium accumulation in maize leaf. *BMC Genomics* 19, 91.
- Zorrig, W., Rouached, A., Shahzad, Z., Abdelly, C., Davidian, J.C., Berthomieu, P., 2010. Identification of three relationships linking cadmium accumulation to cadmium tolerance and zinc and citrate accumulation in lettuce. *J. Plant Physiol.* 167, 1239–1247.
- Zorrig, W., Abdelly, C., Berthomieu, P., 2011. The phylogenetic tree gathering the plant Zn/Cd/Pb/Co P<sub>1B</sub>-ATPases appears to be structured according to the botanical families. *C. R. Biol.* 334, 863–871.
- Zorrig, W., El Khouni, A., Ghnaya, T., Abdelly, C., Davidian, J.C., Berthomieu, P., 2013. Lettuce (*Lactuca sativa*): a species with a high capacity for cadmium (Cd) accumulation and growth stimulation in the presence of low Cd concentrations. *J. Hortic. Sci. Biotechnol.* 88, 783–789.