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Xylem transport and gene expression play decisive roles in cadmium accumulation in shoots of two oilseed rape cultivars (*Brassica napus*)

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HIGHLIGHTS

- L351 accumulates more Cd in shoot rather than intercepting in root than L338.
- Cd uptake by roots was not responsible for the different Cd accumulation in shoots.
- Cd transport by xylem is a critical process determining Cd accumulation in shoots.

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ABSTRACT

Cadmium (Cd) is a toxic metal which harms human health through food chains. The mechanisms underlying Cd accumulation in oilseed rape are still poorly understood. Here, we investigated the physiological and genetic processes involved in Cd uptake and transport of two oilseed rape cultivars (*Brassica napus*). L351 accumulates more Cd in shoots but less in roots than L338. A ~~scanning ion-selective electrode technique (SIET)~~ and uptake kinetics of Cd showed that roots were not responsible for the different Cd accumulation in shoots since L351 showed a lower Cd uptake ability. However, concentration-dependent and time-dependent dynamics of Cd transport by xylem showed L351 exhibited a superordinate capacity of Cd translocation to shoots. Additionally, the Cd concentrations of shoots and xylem sap showed a great correlation in both cultivars. Furthermore, gene expression levels related to Cd uptake by roots (*IRT1*) and Cd transport by xylem (*HMA2* and *HMA4*) were consistent with the tendencies of Cd absorption and transport at the physiological level respectively. In other words, L351 had stronger gene expression for Cd transport but lower for Cd uptake. Overall, results revealed that the process of Cd translocation to shoots is a determinative factor for Cd accumulation in shoots, both at physiological and genetic levels.

1. Introduction

Cadmium (Cd) is a toxic heavy metal without any known physiological function in plants to date (Wang et al., 2007). Cd contamination is widely found in the world due to its release from industrial, urban, and agricultural activities (Kikuchi et al., 2007; Kabata-Pendias, 2010), resulting in human health problems through Cd accumulation from food chains (Obata and Umebayashi, 1997). To reduce the potential risks caused by excessive Cd intake, it is necessary to restrict Cd concentrations in edible plant parts below the threshold values established by the Codex Alimentarius Commission of FAO/WHO (CODEX, 2006).

So far, various strategies have been applied to clean up Cd from polluted soils, such as physical and chemical methods; thermal processes, physical separation, electrochemical methods, washing, stabilization/solidification and burial, which are generally very expensive, and will destroy particle structure and microbial activities in soils (Dermont et al., 2008). Fortunately, phytoremediation, using metal hyperaccumulator plants to remove contaminants from soils, is considered to be a cost-efficient and environmentally friendly remediation technology, despite facing many challenges (McGrath et al., 2006). Another feasible approach for decreasing Cd accumulation in edible plant parts is selecting for low-Cd cultivars by breeding and molecular techniques. However, these methods demand a mechanistic understanding of how Cd is mobilized within plants, both at the physiological and genetic levels (Mendoza-Cózatl et al., 2008). However, little work has been done to date in this area of research, especially for oilseed rape.

Oilseed rapes (*B. napus*, along with *B. juncea* and *B. campestris*) with high homology of the *A. thaliana* genome, are widely grown

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in China. Studies have shown that some oilseed rape cultivars have strong abilities for accumulating Cd into their shoots, and these could be used for phytoremediation of soils contaminated by Cd (Ru et al., 2004).

As reported, there are several physiological processes involved in Cd accumulation in the shoots of plants: uptake by roots, sequestration into root vacuoles, translocation in the xylem and phloem, and questionable dilution within the shoots through growth (Lux et al., 2011). Cd can enter into plant root cells through passive and active transport. The active uptake of Cd by roots is mediated by *ZIP* (Zinc-regulated transporter) and *IRT* (Iron-regulated transporter) (Connolly et al., 2002; Vert et al., 2002; Hussain et al., 2004; Wong and Cobbett, 2009). *AtIRT1* is a primary transporter for iron uptake in *A. thaliana*, and also mediates Cd uptake by roots (Connolly et al., 2002; Vert et al., 2002). Cd loads from symplasm into the xylem by heavy metal P_{1B}-ATPases, such as orthologues of *AtHMA2* and *AtHMA4* (Hussain et al., 2004; Wong and Cobbett, 2009). Further accumulation into seeds or grains is mediated by phloem as illustrated by several studies (Lombi et al., 2000; Greger and Löfstedt, 2004; Uraguchi et al., 2009). However, in oilseed rape, knowledge of the molecular mechanisms responsible for the behaviour of Cd uptake and transport is still poorly reported.

The objectives of the present study were to investigate the physiological and molecular characteristics of Cd uptake by roots and xylem transport in two oilseed rape cultivars with different Cd accumulation levels in shoots. Our data indicated that Cd transport from root to shoot is a critical process determining Cd accumulation in shoots rather than root uptake in these two oilseed rape cultivars. These results show some perspectives for screening/producing crops with low-Cd accumulation in edible plant parts or applying phytoremediation through controlling Cd translocation in plants by physiological and molecular techniques.

2. Materials and methods

2.1. Plant materials and growth conditions

Two oilseed rape cultivars L338 (low Cd) and L351 (high Cd), as evidenced by Cd contents in shoots from 56 oilseed cultivars through solution culture and pot experiments, were used for all experiments. Seeds of L338 and L351 were immersed in deionized water and germinated at 28 °C in the dark. After one week, eighteen uniform seedlings were transferred to a black polyethylene box (52 × 33 × 7 cm) containing 10 L 1/2-strength modified Hoagland solution for 5 days and subsequently full-strength. The full-strength nutrient solution contained 0.5 mmol L^{-1} $NH_4H_2PO_4$, 3 mmol L^{-1} KNO_3, 2 mmol L^{-1} $Ca(NO_3)_2$, 1 mmol L^{-1} $MgSO_4$, 0.09 mmol L^{-1} Na_2EDTA (ethylendiamine-N, N, N, N-tetraacetic acid disodium), 0.09 mmol L^{-1} $FeSO_4$, 22.5 μmol L^{-1} H_3BO_3, 10 μmol L^{-1} $MnSO_4$, 0.10 μmol L^{-1} $(NH_4)_6Mo_7O_{24}$, 0.35 μmol L^{-1} $ZnCl_2$ and 0.20 μmol L^{-1} $CuSO_4$. The pH of the solution was adjusted to 6.7 with 1.0 mol L^{-1} NaOH. The containers were kept in a greenhouse with supplementary condition to maintain a 16 h photoperiod and a temperature controlled at 20–25 °C. The nutrient solution was aerated continuously and renewed once every five days.

2.2. Distribution analysis of Cd in two cultivars

One-week-old seedlings were exposed to modified Hoagland solution containing 0, 0.03 and 0.3 mg L^{-1} Cd. Each treatment was replicated four times. After three weeks, roots and shoots were separated, washed cleanly with deionized water, and dried at 62 °C for 72 h. After weighing, dried plant material (shoot for 0.5 g or root for 0.2 g) was digested with a mixture of HNO_3 and $HClO_4$ (4:1, v/v) with a controlled temperature to 160 °C, the ratio of the volume (mL) of the mixture acid/the mass of tissue (g) was 20:1, and subsequently were subjected to determination of Cd concentrations by flame atomic absorption spectrometry (Z-2000; HITACHI, Tokyo, Japan).

2.3. Analysis of Cd^{2+} flux with the ~~scanning ion-selective electrode technique (SIET)~~

The root hair zones of one-week-old seedlings exposed to modified Hoagland solution were used for measurement of the net Cd^{2+} flux by ~~SIET~~ (BIO-001A; ~~Younger USA Sci. & Tech. Corp.~~, Beijing, China). The Cd ion-selective microelectrodes with an external tip diameter of c. 3 μm were manufactured and silanized with tributylchlorosilane, and the tips were backfilled with a commercially available ion-selective cocktail (Cd Ionophore I, 20909, Fluka, Switzerland). The microelectrodes were calibrated in 50 and 500 μmol L^{-1} Cd^{2+} before the Cd^{2+} flux measurement. The measuring solution contained 0.1 mmol $CaCl_2$, 0.1 mmol L^{-1} KCl, 0.05 mmol L^{-1} $CdCl_2$, 0.3 mmol L^{-1} MES, pH 5.8. The Cd^{2+} flux data were recorded for a period of 0–6 min. The flux data were obtained with the ASET software, which is part of the ~~SIET~~ system and were calculated using MageFlux (http://xuyue.net/mageflux). The negative values in the figure represent the cation influx or anion efflux.

2.4. Kinetic analysis of Cd uptake by roots

For the time-dependent Cd uptake experiment, one four-week-old seedling was transferred to a plastic vessel with 100 mL of modified Hoagland solution containing 0.1 mg L^{-1} Cd. Each vessel represented a individual pot, and was removed during the experiment after each sample was taken. Each treatment was replicated four times. Plants were sampled after 0, 0.5, 1, 2, 4, 6, 8, 10 and 12 h. Shoots and roots were washed cleanly and weighed separately, and the remaining uptake solution was adjusted to 100 mL with deionized water. 3 mL volume of the remaining uptake solution was digested with a 15 mL mixture of HNO_3 and $HClO_4$ (4:1, v/v) with a controlled temperature to 160 °C. Cd concentrations of the measuring solution were determined by graphite furnace atomic absorption spectrometry (Z-2000; HITACHI, Tokyo, Japan).

For the concentration-dependent Cd uptake experiment, one four-week-old seedling was transferred to a black plastic vessel with 100 mL modified Hoagland solution containing different concentrations of Cd (0.01, 0.03, 0.1, 0.3, 1 and 3 mg L^{-1}). Each vessel represented a individual pot, and was removed during the experiment after each sample was taken. Each treatment was replicated four times. After 10 h uptake, Cd concentrations of the uptake solution were determined by graphite furnace atomic absorption spectrometry (Z-2000; HITACHI, Tokyo, Japan) as described in time-dependent Cd uptake experiment.

2.5. Kinetic analysis of Cd transport by xylem

For the time-course Cd transport experiment, eighteen four-week-old seedlings were transferred to a black plastic vessel with 10 L of the modified Hoagland solution containing 0.1 mg L^{-1} Cd, and every three days the solution was changed. Each sampling was replicated three times. Xylem sap was collected at 1 h, 6 h, 1 d, 2 d, 4 d, 8 d and 12 d after Cd exposure started. For collecting xylem sap, plant shoots were cut at 2 cm above the roots and xylem sap was collected from the cut surface for 2 h into a 5 mL vessel. To avoid contamination of symplastic Cd from damaged cells, the initial exudates (1–2 μL) were discarded. The volume of xylem sap was recorded and 2 mL volume of the xylem sap was

digested with a 10 mL mixture of HNO_3 and $HClO_4$ (4:1, v/v) with a controlled temperature to 160 °C, and the Cd concentrations were determined using graphite furnace atomic absorption spectrometry (Z-2000; HITACHI, Tokyo, Japan).

For the concentration-dependent Cd transport by xylem and shoot Cd accumulation experiment, eighteen identical four-week-old seedlings were transferred to a black plastic vessel with 10 L of modified Hoagland solution containing different concentrations of Cd (0.01, 0.03, 0.1 and 0.3 mg L^{-1}) for 9 days. Every three days the solution was changed and each treatment was replicated three times. Xylem sap and shoots were collected. Cd concentrations were determined by graphite furnace atomic absorption spectrometry (Z-2000; HITACHI, Tokyo, Japan) as described in time-dependent Cd transport by xylem experiment.

2.6. Total RNA extraction

One-week-old seedlings exposed to modified Hoagland solution containing three Cd levels (0, 0.03 and 0.3 mg L^{-1}) for three weeks were used for gene expression analysis. Nutrient solution was aerated continuously and renewed every five days. Each treatment was replicated four times. Roots were separated, washed cleanly with deionized water, and stored at −70 °C. For total RNA extraction, frozen root (100 mg) was ground in liquid nitrogen with a pestle. Then, 1 mL of Trizol reagent (Invitrogen, Carlsbad, CA, USA) was added to the sample and mixed. After further addition of 0.2 mL of chloroform, mixing, and centrifugation for 20 min at 4 °C, the superstratum phase was transferred into a new tube. Following addition of isopropyl alcohol to the superstratum phases, the total RNA was deposited and centrifuged for 10 min at 4 °C. After washing with 75% ethanol, the total RNA was dissolved in diethylpyrocarbonate-treated water. RNA quality was evaluated by running on agarose gel. Extracted total RNA was quantified with a Ultrospec 1100 spectrophotometer (Biochrom, England) and stored at −72 °C.

2.7. Quantitative real-time RT-PCR

cDNA was transcribed from total RNA with Oligo(dT) (Promega, Madison, WI USA), M-MLVRTase (Promega, Madison, WI) and dNTP and used as a template for the real-time PCR reaction with an CFX96TM Real-Time PCR Detection System (Bio-Red, USA) using SYBR green for detection of the product at the end of each amplification cycle. Real time-PCR was carried out according to the following cycling program: 30 s denaturation at 95 °C, and then 40 cycles of 5 s at 95 °C, 10 s at annealing temperature of different primer (Table 1) and 15 s at 72 °C. The gene-specific forward and reverse primers and cDNA template were added to the SYBR Green PCR master mix (TOYOBO, Japan). Primers for the genes were designed by Primer Primer 5.0 or OligoArchitect™ Online (http://www.oligoarchitect.com) with gene sequences from GenBank in NCBI. The oilseed rape actin gene was used as a reference for all genes. Primers designed for the genes and reference genes are detailed in Table 1. A dissociation curve was also set up at the end of the 40 cycles in order to ensure that only one product was amplified for each gene. Real-time PCR experiments were conducted on the four biological replicates, with three technical replicates for each sample. The relative quantities of the transcripts were calculated according to (Pfaffl, 2001).

2.8. Statistical analysis of data

All data were statistically analyzed using SPSS 19.0. Analysis of variance (ANOVA followed by LSD multiple comparison) was performed on data sets, with the mean and SE of each treatment calculated.

3. Results

3.1. Distribution analysis of Cd and biomass

As shown in Table 2, the two oilseed rape cultivars L338 and L351 have no significant differences in fresh weight of roots and shoots exposed to the same level of Cd. Shoot Cd concentrations of L351 were 65% and 110% higher than that of L338 under 0.03 and 0.3 mg L^{-1} Cd respectively ($P < 0.01$), while root Cd concentrations of L338 were 104% and 39% higher than that of L351 respectively ($P < 0.01$). The transport coefficients of Cd transport from roots to shoots in L351 were 238% and 200% higher than that of L338 under 0.03 and 0.3 mg L^{-1} Cd respectively ($P < 0.01$). Shoot Cd accumulation of L351 was 71% and 86% higher than that of L338 under 0.03 and 0.3 mg L^{-1} Cd respectively ($P < 0.01$), while root Cd accumulation of L338 was 88% and 27% higher than that of L338 respectively ($P < 0.01$). The ratio of shoot Cd accumulation/root Cd accumulation in L351 was 220% and 138% higher than of L338 under 0.03 and 0.3 mg L^{-1} Cd respectively ($P < 0.01$). No significant differences were observed in total Cd accumulation between the two cultivars under two Cd treatments. These results show that L351 accumulates more Cd in shoots rather than intercepting in roots compared with L338.

3.2. Net fluxes of Cd^{2+} by roots

The ~~scanning ion-selective electrode technique (SIET)~~ was used to compare the different Cd^{2+} influxes of two oilseed rape cultivars (L338 and L351). As shown in Fig. 1B, the net Cd^{2+} influx of L338 was 68% higher than that of L351 ($P < 0.01$), indicating than L338 has greater effective uptake for Cd.

3.3. Kinetic analysis of Cd uptake by roots

Time-dependent and concentration-dependent Cd uptake experiments using intact seedlings were conducted to evaluate the differences of Cd uptake abilities by roots of two oilseed rape

Table 1
Sequences of primers used for RT-PCR.

Target	Primer sequences (5′–3′)	Annealing temperature	Amplification efficiency	Gen bank accession no.
BnActin2.1	F-CTCTTTCACACGCCATCCTCC R-GATTCCAGCAGCTTCCATTCC	57.5 °C	94%	FJ529167.1
AtIRT1	F-AATGTTGTCATCTATATGTCTTG R-CGTTCTTGCTGGTGTATA	57.5 °C	104%	NM_179215.1
AtHMA2	F-TTGAACCTAAGCCTGAAG R-TCTCCAACATATACATATCCAA	63 °C	92%	NM_119157.3
BjHMA4	F-CAAGTAGTAAGTCCAGTTCT R-GATGAAGCCATATCTCCAA	49 °C	97%	JQ673430.1

Note: 'F' was defined as forward primer, and 'R' as reverse primer.

Table 2
Biomass, Cd concentrations and Cd accumulation in tissues of L338 and L351.

	Cd 0 mg L^{-1}		Cd 0.03 mg L^{-1}		Cd 0.3 mg L^{-1}	
	L338	L351	L338	L351	L338	L351
Shoot DW (g $plant^{-1}$)	1.19 ± 0.12	1.25 ± 0.11	1.15 ± 0.10	1.21 ± 0.13	0.95 + 0.01	0.92 ± 0.01
Root DW (g $plant^{-1}$)	0.30 ± 0.02	0.34 ± 0.03	0.31 ± 0.02	0.33 ± 0.03	0.29 ± 0.02	0.31 + 0.02
Shoot Cd conc. (μg g^{-1} DW)	nd	nd	4.29 ± 0.45	7.06 ± 0.58**	23.76 ± 2.17	49.80 ± 3.53**
Root Cd conc. (μg g^{-1} DW)	nd	nd	32.52 ± 2.72**	15.98 ± 0.12	179.12 ± 18.28**	128.83 ± 10.64
Transport coefficient	–	–	0.13 ± 0.01	0.44 ± 0.03**	0.13 ± 0.01	0.39 ± 0.04**
Shoot Cd accum. (μg $plant^{-1}$)	nd	nd	4.94 ± 0.27	8.44 ± 1.02**	24.54 ± 3.46	45.76 ± 1.78**
Root Cd accum. (μg $plant^{-1}$)	nd	nd	9.93 ± 0.94**	5.27 ± 0.41	52.19 ± 7.51**	40.98 ± 4.31
Total Cd accum. (μg $plant^{-1}$)	–	–	14.87 ± 1.06	13.71 ± 1.33	76.73 ± 10.23	86.74 ± 5.64
Relative Cd accum. (shoot/root)	–	–	0.50 ± 0.04	1.60 ± 0.11**	0.47 ± 0.05	1.12 ± 0.09**

Data are the means of four replicates (±se). * and ** indicate separation between the two oilseed rape cultivars at the same Cd treatment by ANOVA followed by LSD multiple comparison (*: $P < 0.05$, **: $P < 0.01$). 'nd' indicates 'not detected'. Transport coefficient indicates as the ratio of shoot Cd concentration/root Cd concentration, and relative Cd accumulation as the ratio of shoot Cd accumulation/root Cd accumulation.

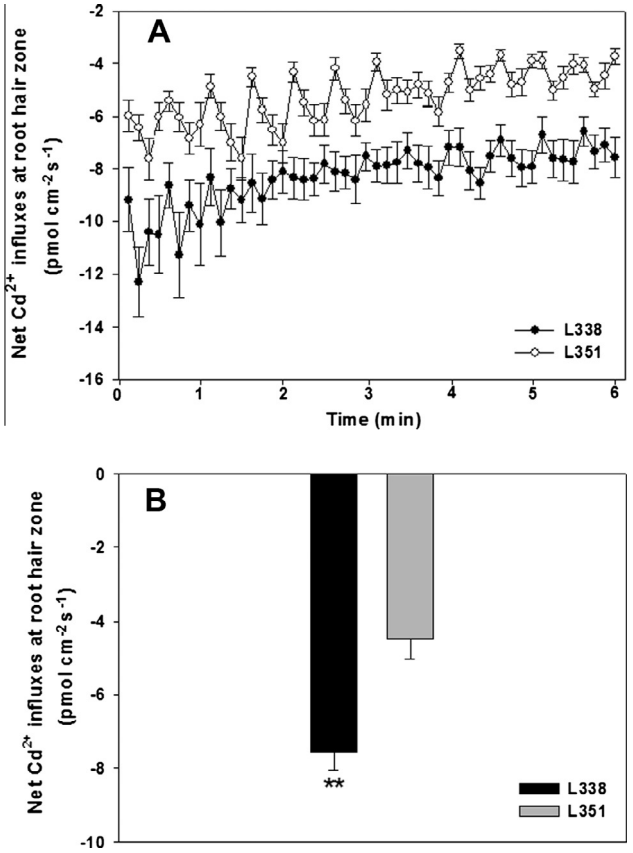

Fig. 1. Net Cd^{2+} influxes measure by scanning ion-selective electrode technique of L338 and L351 (A, B). Each point represents the mean of four roots from four individual plants; the bars represent the standard error of the mean (A). Data are the means from the time of 4th–6th minute for B only, * and ** indicate separation between the two oilseed rape cultivars at the same time and the same Cd treatment by ANOVA followed by LSD multiple comparison (*: $P < 0.05$, **: $P < 0.01$).

cultivars (L338 and L351). As shown in Fig. 2A, the time-dependent experiment of L338 and L351 showed that the pattern of Cd uptake by roots displayed an initial, rapid linear stage during the first 1 h, followed by a second, slower curvilinear stage over the subsequent 8 h and a saturated stage for the last two hours, and was 13–78% higher in L338 than that of L351 during the 12 h treatment. The Cd uptake rates of L338 and L351 reached maximum at 1 h and 0.5 h respectively, and subsequently decreased to the saturated stage. The Cd uptake rate was significantly higher in L338 than in

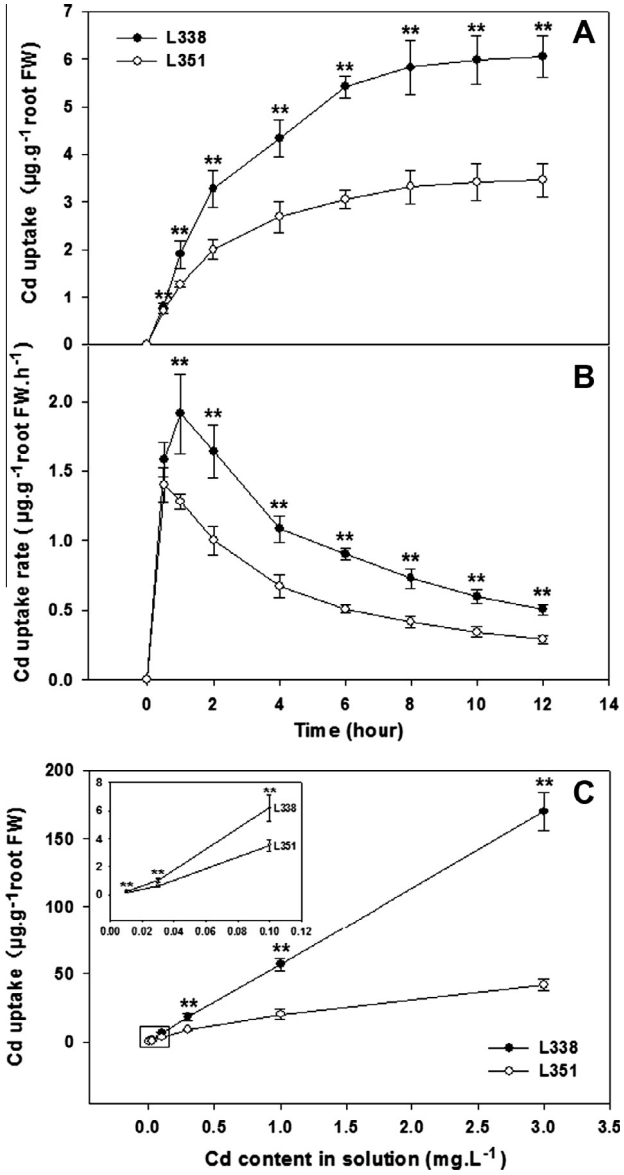

Fig. 2. Time dependency Cd uptake (A) and Cd uptake rates (B), and concentration dependency Cd uptake (C) by roots of L338 and L351. Data in C were taken after 10 h of exposure to Cd. Data are the means of four replicates (±se). * and ** indicate separation between the two oilseed rape cultivars at the same time or the same Cd treatment by ANOVA followed by LSD multiple comparison (*: $P < 0.05$, **: $P < 0.01$).

L351 ranging from 49% to 78% at individual times, except for 0.5 h after Cd exposure (Fig. 2B, $P < 0.01$).

Further investigation with the concentration-dependency Cd uptake by roots of L338 and L351 show linear concentration-dependency with $R^2 = 0.99$ and $R^2 = 0.97$ respectively, and was much higher in L338 than in L351 at each Cd level being 65–303% higher as the Cd concentrations increased from 0.01 to 3 mg L^{-1} (Fig. 2C, $P < 0.01$).

These results suggested that the Cd uptake ability by roots in L338 is superior to that of L351 under the present experimental conditions.

3.4. Analysis of Cd transport by xylem and Cd accumulation in shoots

To clarify the differences of Cd translocation by xylem and Cd accumulation in shoots of two oilseed rape cultivars (L338 and L351), Cd uptake kinetics by xylem and Cd accumulation in shoots were analyzed. In the time-dependent experiment, the Cd concentrations in xylem sap in both cultivars came to maximum values at 1 d after Cd exposure started, and subsequently decreased slightly to saturated levels (Fig. 3A). Only in L351, the Cd concentrations in xylem sap always exceeded the external Cd concentration of 0.1 mg L^{-1} (75% higher), and were maintained at certain levels 8 d from the start of Cd treatment. The Cd concentration in xylem sap of L351 was remarkably higher in contrast with that of L338 ranging from 61% to 195% higher at each individual sampling period (Fig. 3A, $P < 0.01$).

For the concentration-dependent Cd transport by xylem, the Cd concentrations in xylem sap exhibited a concentration-dependent rise in both cultivars after 9 d exposure to Cd, and were significantly greater in L351 than that in L338 ranging from 55% to 145% higher at different levels of Cd (Fig. 3B, $P < 0.01$). The shoot Cd concentrations also showed a concentration-dependent pattern in both cultivars, and were significantly higher in L351 with the values ranging from 65% to 90% higher (Fig. 3C, $P < 0.01$). The Cd concentrations in shoots were nearly linear growing with increasing of the Cd concentrations in xylem sap with high correlations in both cultivars ($R^2 = 0.95$ and 0.91 for L338 and L351 respectively, Fig. 3D) though no significant differences were found between low Cd concentrations (0.01 and 0.03 mg L^{-1}). These results demonstrated that L351 has a greater Cd transport ability by xylem than L338, leading to greater Cd accumulation in shoots.

3.5. Relative expression analysis of genes related to Cd uptake and transport

To investigate the differences of Cd uptake and transport at genetic level, we examined the changes of relative expressions of *IRTI*, *HMA2* and *HMA4* genes in roots of two cultivars exposed to three Cd levels. *IRT1* expression in roots of L338 was 46% ($P < 0.01$) and 92% ($P < 0.01$) higher than those of L351 under 0.03 mg L^{-1} and 0.3 mg L^{-1} Cd respectively. No significant differences were observed between the two cultivars at 0 mg L^{-1} Cd (Fig. 4A).

HMA2 and *HMA4* expression of L351 were 66% ($P < 0.01$) and 12% higher than those of L338 at 0.03 mg L^{-1} Cd treatment, and 50% ($P < 0.01$) and 34% higher ($P < 0.01$) at 0.3 mg L^{-1} Cd level (Fig. 4B and C). Results indicated that L338 has a higher ability of

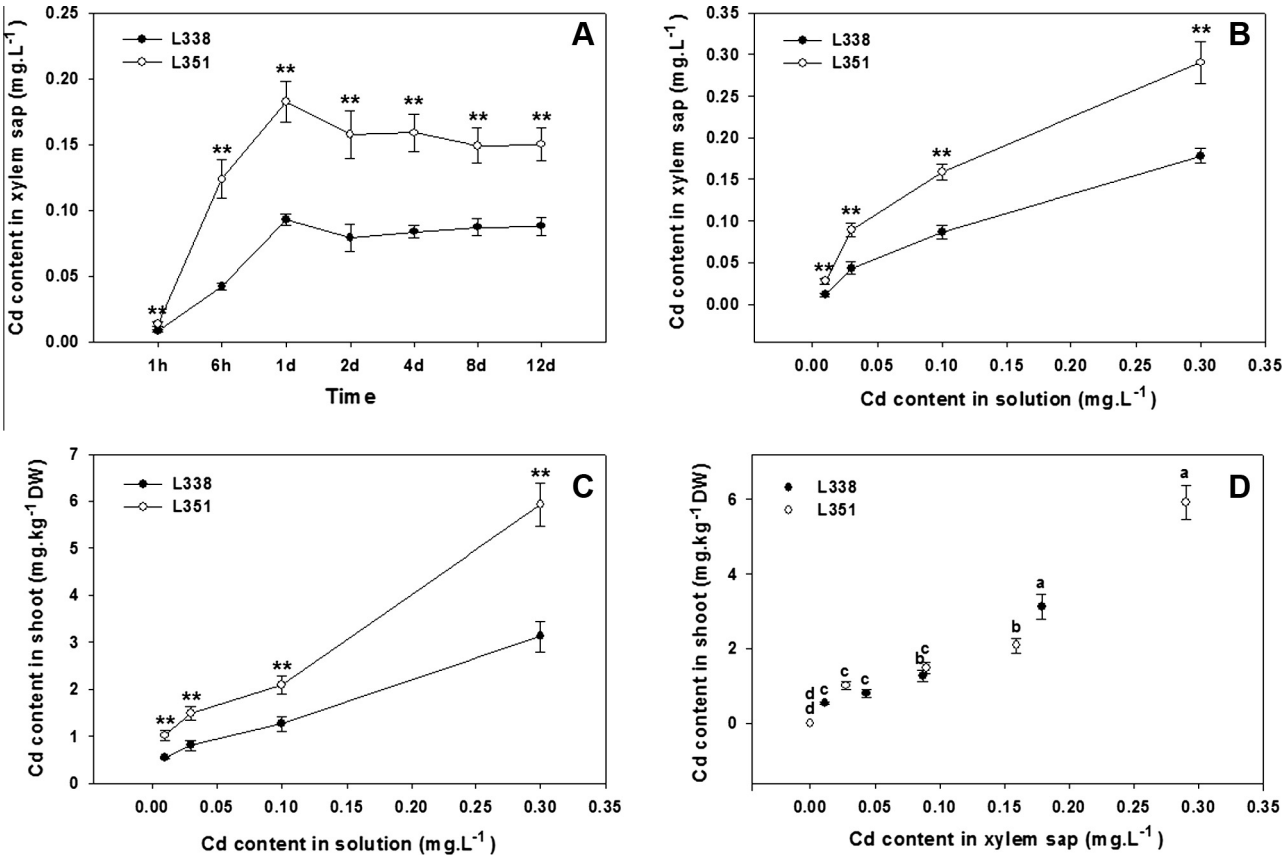

Fig. 3. Time dependency (A) and concentration dependency (B) of Cd concentrations in xylem sap, and concentration dependency of Cd concentrations in shoots (C), and correlative analysis (D) between L338 and L351. Values (D) followed by the different letters indicate significant differences based on one-way ANOVA followed by LSD test ($P < 0.05$) for each cultivar. Data are the means of four replicates (±se). * and ** indicate separation between the two oilseed rape cultivars at the same time or the same Cd treatment by ANOVA followed by LSD multiple comparison (*: $P < 0.05$, **: $P < 0.01$).

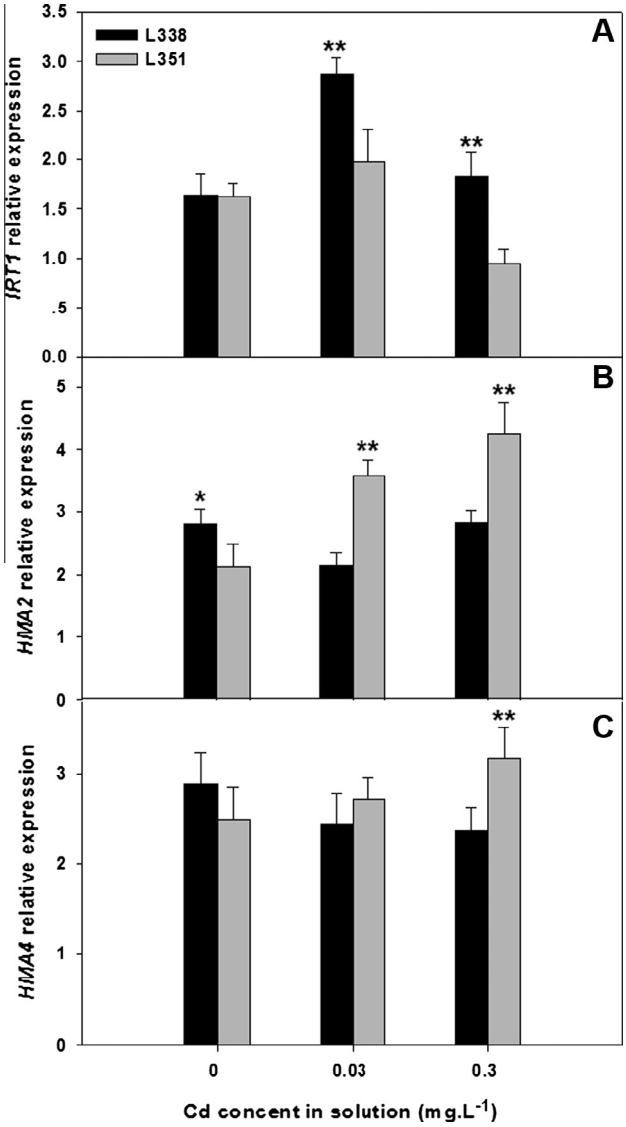

Fig. 4. *IRT1* (A), *HMA2* (B) and *HMA4* (C) expression levels in roots of L338 and L351. Data are the means of four replicates (±se). * and ** indicate separation between the two oilseed rape cultivars at the same Cd treatment by ANOVA followed by LSD multiple comparison (*: $P < 0.05$, **: $P < 0.01$).

gene expression involved in Cd uptake but lower in transport compared with L351 at the mRNA transcription level.

4. Discussion

4.1. Growth response and Cd accumulation in tissues of two oilseed rape cultivars

As reported, desired hyperaccumulator plants have several beneficial traits: (1) fast growth and high biomass; (2) good tolerance and high concentrations of metals in shoots; (3) high transport coefficient, defined as the ratio of the metal concentration in shoot to that in root (Vamerali et al., 2010). *Brassica* species are well known as metal accumulators and have been investigated for several years for the accumulation ranges of metals in shoots (Blaylock, 2000; Ghosh and Singh, 2005). Biomass of roots and shoots is indicated as an important index to evaluate plant tolerance to Cd toxicity. In our study, L338 and L351 have no significant differences in fresh weights of shoots and roots under the same Cd treatment (Table 2). This data indicated that the two oilseed rape cultivars have the same Cd tolerance exposure to Cd. Additionally, Cd concentrations in shoots and the transport coefficient in L351 were much higher than that of L338 (Table 2). These tendencies were also found in Cd accumulation in shoots and the ratio of shoot Cd accumulation/root Cd accumulation, though no significant differences were observed in the total Cd accumulation between the two cultivars (Table 2). These results suggested that in *B. napus*, the cultivar L351 has a higher Cd accumulation tendency compared to L338.

4.2. Cd uptake and transport into shoots

As is known, several physiological processes may contribute to high Cd accumulation in shoots, including efficient roots uptake and/or efficient xylem transport (Ueno et al., 2011). Cd can enter into plants root cells by apoplastic binding and symplastic uptake (Hart et al., 1998). Large variations in Cd distribution between roots and shoots were found in different genotypes of the same species when grown in the same condition (Metwally et al., 2005). As reported, the differences in Cd accumulation in shoots were mainly ascribed to absorption abilities by roots (Lombi et al., 2000). On the other hand, Hart concluded that Cd accumulation in the grains between durum wheat and bread wheat might be ascribed not only to root uptake and xylem transport but also to phloem-mediated translocation to the grain. In our study, L338 which has a low Cd concentration in shoots, showed a higher capacity for Cd uptake and rate by roots in comparison with that of L351 (Figs. 1A and B and 2A–C). These results presented here indicated that Cd uptake by roots is not a critical reason for the divergent differences in Cd accumulation of shoots between L351 and L338, and these were consistent with the previous studies (Greger and Löfstedt, 2004; Uraguchi et al., 2009; Ueno et al., 2011). Furthermore, it is generally believed that the *ZIP* (zinc regulated transporter) and *IRT1* (iron regulated transporter) are the best-studied nonspecific transporters responsible for iron uptake by roots, and also mediate Cd uptake or other bivalent heavy metals (He et al., 2006). Evidence from a previous study indicated that Cd and Zn partially shared the same transport pathway as Zn inhibited Cd uptake by 38.55% and 30.42% for mutant and wild type rice respectively (He et al., 2008). In *A. thaliana, AtIRT1* has been identified for mediating Cd uptake from soils, and could be regulated by overexpression or deletion mutation (Korshunova et al., 1999; Rogers et al., 2000). Here, L338 had higher expression of *IRT1* than that of L351 in root (Fig. 4A), and this may be one reason to explain higher Cd uptake by roots.

After being absorbed by roots, Cd can rapidly be transported into shoots by xylem, and this is suggested as a next important process for metal accumulation in shoots of plants (Clemens et al., 2002). In our study, although root Cd uptake ability was not superior in L351 compared to that of L338, more rapid and higher root-to-shoot translocation of Cd by xylem was observed in L351 (Fig. 3A–C). As a result, a high proportion of Cd uptake by roots was transported to shoot in L351, while L338 detained most of Cd in roots. In addition, the Cd concentrations in shoots and xylem sap exhibited a great correlation in both cultivars (Fig. 3D), which was also observed in research of 69 rice varieties in a field experiment (Uraguchi et al., 2009). These results demonstrate that the differences in Cd accumulation between the two oilseed rape cultivars are due to different efficiency of Cd translocation by xylem. Similar results have also observed that Cd transport from roots to shoots is a key factor determining Cd accumulation in shoots (Harris and Taylor, 2004; Hart et al., 2006; Mori et al., 2009; Uraguchi et al., 2009; Yamaguchi et al., 2011).

Recently, many studies have demonstrated that P_{1B}-type heavy metal ATPases, *HMA2* and *HMA4*, which are located at the plasma membranes and expressed predominantly in the root vascular cells, play important roles in root-to-shoot translocation of Cd in plants. In *A. thaliana*, *AtHMA2* and *AtHMA4* were found to be required for the root-to-shoot translocation of Cd and Zn (Hussain et al., 2004; Wong and Cobbett, 2009). Overexpression of *AtHMA4* enhanced root-to-shoot translocation of Cd and Zn and metal tolerance (Verret et al., 2004), whereas disruption of *AtHMA4* function results in increased sensitivity to Cd and Zn in *A. thaliana* (Mills et al., 2005). Similar research has also been reported in other plants (Hanikenne et al., 2008; Siemianowski et al., 2011; Takahashi et al., 2012). In addition, (Ueno et al., 2011) reported that loss of function of *OsHMA3* is also responsible for high Cd accumulation in rice. In the present study, L351 showed greater ability of expression in *HMA2* and *HMA4*, especially for *HMA2*, leading to more Cd accumulation in shoot (Fig. 4B and C).

In conclusion, results from the present study show that efficient root-to-shoot translocation of Cd is responsible for high Cd accumulation in L351, both at physiological and molecular levels.

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