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Full Length Article

Comparative Study on Physiological and Genetic Processes Involved in Cd uptake and Transport of *Brassica juncea* and *Brassica napus* in Response to Cd Stress

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Abstract

Soil cadmium (Cd) pollution not only affects crop yields and quality, but also endangers human health through the food chain. *Brassica napus* (*B. napus*) and *Brassica juncea* (*B. juncea*) are important oilseed crops. Obtaining good oil crop cultivars capable of enriching heavy metals and producing oil and is of great significance for soil heavy metal pollution restoration. *B. napus* and *B. juncea* were treated with 0, 50 and 200 mg kg^{-1} Cd for 30 days at the initial flowering stage, respectively. Results indicated that *B. napus* accumulates more Cd in shoots than *B. juncea* and the biological enrichment factor (BCF) was higher than that of *B. juncea* under 50 mg kg^{-1} Cd stress. The translocation factor (TF) of *B. napus* was also higher than that of *B. juncea* under 50 mg kg^{-1} Cd stress. Additionally, the total percentage of oleic acid and linoleic acid in grains, which are beneficial to human health, were slightly reduced in *B. napus*. Moreover, Cd stress interfered with mineral nutrients absorption, transport and redistribution. In addition, most of the candidate genes in *B. na*pus were up-regulated, and down-regulated in *B. juncea* under Cd stress condition. Therefore, *B. napus* was more tolerant to Cd stress. In conclusion, findings of this study may provide theoretical basis and direction for better understanding the mechanism of Cd uptake and transport.
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Keywords: Cadmium; Hyperaccumulation; Translocation; Mineral nutrients; *B. napus; B.juncea*

Introduction

The heavy metal cadmium (Cd) is widely distributed in the natural environment (Lalor *et al.* 2004). Although Cd is not a necessary element for plants, can readily absorb by plants because of the similarity with many divalent metal ions such as zinc, iron, and calcium (Pottier *et al.* 2015). Once it enters the plant, Cd may depress the growth and yield of plants (Wang *et al.* 2013; Naggar *et al.* 2014). Additionally, Cd was able to be accumulated in the edible portions of plants, especially in crops. Because Cd is migratory along the food chain, it may pose potential risks to humans and other animals (Clemens *et al.* 2013).

Over the last decade, Cd-contaminated soil has been increased dramatically (Chibuike and Obiora 2014). Hence, it is essential to conduct Cd-contaminated soil restoration. Phytoremediation, as an environment-friendly and sustainable remediation method for contaminated soils, has attracted wide attention (Pilon-Smits and Freeman 2006; Rosa *et al.* 2014). However, the uptake, transport, accumulation and redistribution of Cd from soil to other tissues of plants require different transporters, for example the ZIP family (ZRT: zinc regulated transporters, IRT-like protein: iron-regulated transporter-like protein); the HMA family (heavy metal ATPase transporters); the MATE family (multidrug and toxic compound extrusion protein transporters); the NRAMP family (natural resistance-associated macrophage protein); the ABC family (ATP-binding cassette proteins); and the oligopeptide transporters family and so on (Nevo and Nelson 2006; Sasaki *et al.* 2016; Wu *et al.* 2016). Transporters are essential for Cd absorption, translocation and distribution in plants. To under the molecular mechanisms of Cd transport process and the role of transporters is helpful to the development of hyperaccumulators. The ideal plant for using in phytoremediation should grow fast, high biomass, preferably in the aboveground parts, ability to absorb and tolerate high metal concentrations (Baker and Whiting 2002; Whiting 2010; Goswami and Das 2015). At present, the

heavy metal hyperaccumulators such as the *Noccaea caerulescens* and the *Arabidopsis halleri* screened have the capacity for Cd accumulation, but most of them are slow-growing, the aboveground biomass is low, which is not conducive to large-scale mechanization. It is common knowledge that *Brassicaceae* are the heavy metal accumulators, the accumulation ranges of heavy metals in shoots have been studied for many years (Ghosh and Singh 2005; Flores-Caceres *et al.* 2015). Indian mustard (*B. juncea*) and *B. napus* belong to *Brassicaceae* family and the former is considered to be one of the most potential phytoremediation species, because of their higher biomass and higher heavy metal accumulating capacity in the shoot (Eapen and D'Souza 2005; Goswami and Das 2015). However, the oil production of *B. juncea* is very low and its growth area is very limited. It is difficult to perform large-scale planting of *B. juncea* in China. *B. napus*, as an important oil crop, has larger biomass and higher oil production compared to *B. juncea*. It is popularly planted in the middle and lower reaches of the Yangtze River in China. Because of its prolific growth, *B.napus* can be grown advantageously for phytoremediation (Meng *et al.* 2008). Hence, researching the physiological and genetic processes of Cd uptake, transport and accumulation in *B. juncea* and *B. napus*, are beneficial to the improvement of Cd tolerance and resistance in plant, further controlling the migration process of Cd in food chain.

In this study, one potential Cd accumulation cultivar of *B. napus* and the normal species of *B. juncea* were selected and compared under different Cd stress conditions. A comprehensive investigate on the biomass, physiological characteristics, Cd and mineral element enrichment ability and grain quality, subsequent the genetic processes of Cd uptake and transport at different Cd concentrations were studied. The current research is very important to find out the differences between the two *Brassica* species in response to Cd stress, obtaining an oilseed rape species that can enrich Cd without affecting its economic value, finally providing a theoretical basis for the screening of practical Cd hyperaccumulators in response to agricultural Cd-contaminated soil.

Materials and Methods

Plant materials and treatment

The pot experiment was conducted at Southwest University, Beibei District, Chongqing, China (106.4 N, 29.8E). The tested soil in the experimental consisted of a typical peat (Klasmann-Deilmann, pH6.0, no Cd, Germany) and vermiculite (diameter 3–6 mm). Seeds of *B. napus* (Zhongyou 821) and *B. juncea* (Ping shan qing cai) were obtained from the College of Agronomy and Biotechnology of the Southwest university. Seeds of *B. napus* and *B. juncea* were surface sterilized using 6% (w/v) NaClO (including 0.05% Twain20) for 3 min, rinsed completely in sterile water and incubated in petri dishes. The seeds were then vernalized at 4°C for 14 days in the darkness and then grown in an culture room with 16 h light (25°C, 5000Lux) and 8 h dark (20°C) photoperiod. After 8 days for germination, uniform seedlings of Brassica were transferred into plastic pots (25×33 cm) containing 2 kg of tested soil, one plant per pot and watered every three days. The experiments were carried out in the greenhouse of Southwest University under normal illumination and temperature conditions (the winter temperature ranges from 0 to 10°C necessary for vernalization).

After approrimately 100 days growth, one week before the initial flowering stage, the plants were exposed to the soils with 0, 50 and 200 mg kg^{-1} Cd stress for 30 days, eight repetitions per treatment.

The first sampling was carried out after 30 days of Cd treatment. Four repetitions of different Cd treatments were harvested. At harvest, plants were divided into roots (R), lower stems (LS), lower leaves (LL), upper stems (US), upper leaves (UL) and siliques (S). The leaves of some samples were taken out, immediately frozen in liquid nitrogen and then stored at -80°C for RNA extraction. All other tissues were dried in oven at 70°C until they reached constant mass and weighed for biomass determination. And the rest of plants were harvested when the siliquesss had fully matured and the leaves had senesced. Corning peeled to leave rapeseed for Cd content and determination of fatty acid components.

Mineral nutrients contents

The sample of oven-dried was crushed and sieved (60 mesh) for mineral nutrients detection. The contents of Cadmium (Cd), Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn), Iron (Fe) and Manganese (Mn) in plant tissues were measured via Flame Atomic Absorption Spectrometry (FAAS) after digestion of the plant samples with diacid (3 HNO_3: 1 $HClO_4$; v/v).

Fatty acid components

Fatty acid components of seeds, removed from the siliques, was detected by GC analysis (GC-2010, Shimadzu, Japan) as mentioned by Lian (Lian *et al.* 2017). In all of 200 mg dried rapeseed were crushed and then transferred to 5 mL glass tube and added 2 mL petroleum ether and ether solution (1:1), then the sample was shaken gently. After dissolving (about 40 min), 1 mL KOH-methanol solution (0.4 mol/L) was added to the sample, and then the mixture was mixed to carry out methyl esterification (about 30 min at the room temperature). Added 2 mL distilled water, mixed and shaken. After demixed, taking 1 mL of supernatant to the GC tube for detected.

The chromatograph column type is a DB-WAX (30 m × 0.246 mm × 0.25 ppm). The stationary phase was

polyethylene glycol. The column temperature was 185°C, the vaporization temperature was 250°C, and the detector temperature also was 250°C. The gas flow rate of nitrogen (carrier gas) was 60 mL/min, hydrogen was 40 mL/min and air was 400 mL/min. 2 uL sample was injected and the peak retention time was 13 min. According to the meteorological chromatogram of each sample to be measured, the peak area normalization method is used to calculate the percentage of each fatty acid component.

Photosynthetic efficiency

After 30 days of Cd treatment, the shoot of plant was divided into two layers with the third long-handled leaves at the under of lowest effective branch. Photosystems activity parameters were all detected in Portable photosynthesis systems (CIRAS2) between 10:00 to 11:00 at Sunny morning.

RNA isolation, complementary DNA (cDNA) synthesis and real-time quantitative-PCR

The EZ-10 DNAaway RNA Mini-prep Kit (Sangon, Shanghai) was used to extract the total RNA from frozen upper leaves of *B. napus* and *B. juncea* according to the manufacturer's protocol. The concentration and quality of RNA samples have been tested by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa, Tapan) was used for the cDNA synthesis.

Real-time quantitative-PCR was used to identify the expression level of Cd transport related genes for which homologous to *A. thaliana* had been reported. All genome sequences were blast and downloaded from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). All primers (Additional file 2: Table S2) for RT-qPCR were designed based on tool Primer3 (http://bioinfo.ut.ee/primer3–0.4.0/primer3/) and synthesized commercially (BGI, Beijing, China). All the expression data were obtained from three individual biological replicates and three independent technical replications for each cDNA template. The samples were standardized as the selected internal reference gene and the expression levels of related genes were calculated by the 2^{-DDCt} method.

Statistical analysis

The SPSS 18.0 and GraphPad Prism 5 were used to statistically analyze all data. The data sets were analyzed by variance analysis to calculate the mean values and SD of each treatment. The Duncan's multiple range test was used for significant analysis of differences between groups of samples ($p < 0.05$). Student's t test was used to compares two means and the significant difference between two groups ($p < 0.05$).

Results

The effects of Cd stress on the biomass in the two brassica species

The dry weight of roots and stems of *B. napus* and *B. juncea* were decreased remarkably under Cd treatment (Fig. 1A and B). The 50 mg kg^{-1} Cd treatment significantly affected the dry weight of leaves of *B. napus* and *B. juncea*. The dry weight of *B. napus* leaves was slightly reduced, while that of *B. juncea* was increased, under 200 mg kg^{-1} Cd treatment condition (Fig. 1C). On the contrary, with the increase of Cd concentration, the dry weights of siliques of *B. napus* and *B. juncea* were also increased, respectively. When the Cd concentration was up to 200 mg kg^{-1}, the dry weight of siliques of *B. napus* was up to 2.46 g twice as much as that under normal conditions (Fig. 1D). However, the biomass of *B. napus* was weakly decreased under 50 mg kg^{-1} Cd treatment condition (Fig. 1E). The root/shoot ratio of *B. napus* was almost unaffected by Cd treatments. However, the root/shoot ratio of *B. juncea* was significantly decreased under Cd treatment condition. The results suggested that the Cd stress had no significant effect on the biomass of both *B. napus* and *B. juncea* after 30 days of Cd treatment at flowering stage. Comparably, *B. juncea* was more sensitive to Cd treatment when comparing with *B. napus*.

The effect of Cd stress on photosynthetic efficiency

The Cd treatment weakly decreased the Ci in UL of *B. napus*. The Ci in LL of *B. napus* and *B. juncea* were not affected by Cd treatment, respectively (Fig. 2A). The Tr in UL of *B. napus* was improved by Cd treatments, while the Tr in LL was weakly reduced under 50 mg kg^{-1} Cd treatment condition. The effect of Cd stress on Tr of *B. juncea* was consistent with that of *B. napus* (Fig. 2B). The Gs in both ULand LL of *B. napus* were reduced under 50 mg kg^{-1} Cd treatment condition, in contrast, the Gs in both ULand LL of *B.napus* were increased under 200 mg kg^{-1} Cd treatment condition. Moreover, the Gs of UL and LL of *B. napus* were 699ppmol/m^2/s and 623ppmol/m^2/s, which was 1.4 times and 1.5 times higher than that in the normal condition, respectively. The Gs in UL and LL of *B. juncea* were weakly increased (Fig. 2C). The Pn in both UL and LL of *B. napus* were reduced under 50 mg kg^{-1} Cd treatment condition, and then were increased under 200 mg kg^{-1} Cd treatment condition. The effect of Cd treatment on the Pn in UL of *B. juncea* was consistent with that of *B. napus*, but had no obviously effect on the Pn in LL of *B. juncea* (Fig. 2D). The results revealed that the photosynthesis of lower leaves (LL) which were also older leaves, in both Brasscia species, especially in *B. juncea*, was more significantly affected by Cd stress compared with the upper leaves (UL), which were also younger leaves.

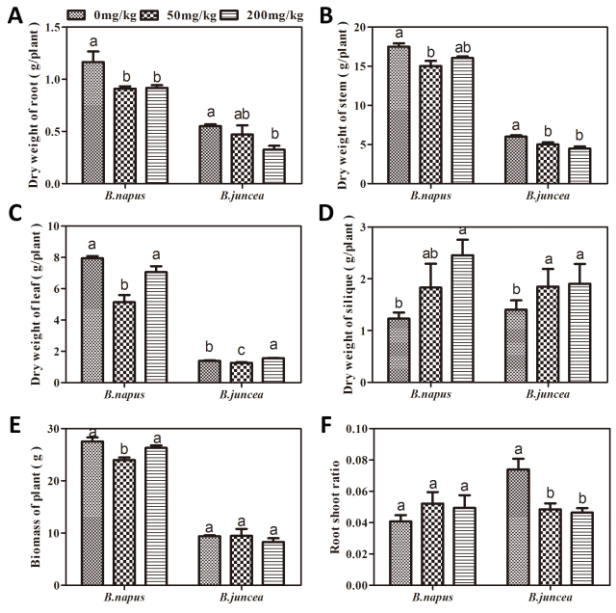

Fig. 1: Effects of different Cd concentrations on dry weights of root (A), stem (B), leaf (C), silique (D), whole plant (E) and the root/shoot ratio (F) of two Brassica species

Data were statistically evaluated using Duncan's test ($P < 0.05$). At least four biological replicates were performed for each treatments

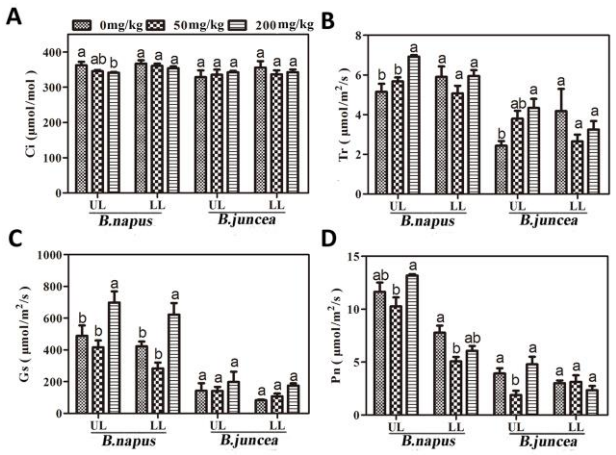

Fig. 2: The effect of Cd stress on Ci: Intercellular CO_2 concentration (A), Tr:Transpiration (B), Gs: stomatal conductance (C) and Pn: Variation of net CO_2 assimilation (D) of the two Brassica species

Data presented are the means (n=4), and error bars denote the standard deviations. Data were statistically evaluated using Duncan's test ($P < 0.05$)

The effects of different Cd concentrations on the Cd contents of different organs of the two brassica species

The result showed that higher Cd stress could increase Cd uptake and accumulation activities in *B. napus* and *B. juncea* (Fig. 3). Interestingly, the Cd contents in *B. napus* were mostly higher than that in *B. juncea* under different Cd treatments (Fig. 3A–F), except for the Cd contents in the seeds under 200 mg kg^{-1} Cd treatment condition (Fig. 3E).

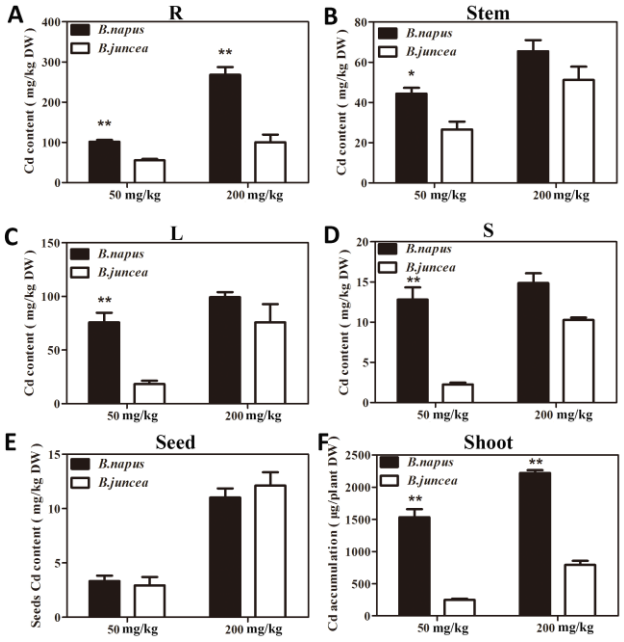

Fig. 3: Effects of different Cd concentrations on the Cd contents ofroots (A), stems (B), leaves (C), siliques (D), seeds (E) and Cd accumulation of shoot (F) of the two Brassica species

Data were statistically evaluated using Student's t test ($P < 0.05$). At least four biological replicates were performed for each treatments

The accumulation of shoot in *B. napus* was higher than that in *B. juncea* (Fig. 3F). Under 50 mg kg^{-1} Cd stress, the accumulation of shoot in *B. napus* were up to 1532.30 *μ*g/plant, were 6.13 times to that of *B. juncea*, respectively. Under 200 mg kg^{-1} Cd stress, the accumulation of shoot in *B. napus* were up to2219.27 *μ*g/plant, which were 2.80 times to that of *B. juncea*. The result revealed that *B. napus* can significantly accumulate more Cd comparing with *B. juncea* under same Cd treatment conditions. Therefore, the cultivar of *B. napus* chosen in the present study is an potential species for agricultural soil heavy metal remediation in the future.

The effects of Cd stress on the uptake, transport and accumulation

The BCF is the ratio of Cd concentration in plant tissues to exogenous Cd. TF is the ratio of Cd concentration in shoot to that in root. Therefore, higher the BCF value, the stronger was cadmium accumulation ability. Likewise, higher the TF value, the higher was cadmium transfer ability from root to shoot. Table 1 showed that the $BCF_{root/soil}$, $BCF_{lower\ stem/soil}$, $BCF_{lower\ leaves/soil}$, $BCF_{upper\ stem/soil}$, $BCF_{upper\ leaves/soil}$, $BCF_{siliques/soil}$ of *B. napus* were decreased when the Cd concentration increased from 50 to 200 mg kg^{-1} and the $BCF_{root/soil}$, $BCF_{lower\ stem/soil}$, $BCF_{lower\ leaves/soil}$, $BCF_{upper\ stem/soil}$, $BCF_{upper\ leaves/soil}$, $BCF_{siliques/soil}$ were reduced by 38.81, 58.88, 69.90, 57.14, 69.70 and 72%, respectively. The $BCF_{root/soil}$, $BCF_{lower\ leaves/soil}$ and $BCF_{upper\ stem/soil}$ of *B. juncea* were decreased when the Cd concentration increased from 50 to

Table 1: Biological enrichment factor (BCF) and Translocation factor (TF) values of different Cd concentration of the two oilseed rape cultivars

Cd (mg kg^{-1})	Cultivars	$BCF_{root/Soil}$	$BCF_{lowerstem/Soil}$	$BCF_{lowerleaves/Soil}$	$BCF_{upperstem/Soil}$	$BCF_{upperleave/Soil}$	$BCF_{Silique/Soil}$	TF
50	*B.napus*	2.19±0.15 **	1.07±0.05 **	1.96±0.39 *	0.70±0.05	1.32±0.32 *	0.25±0.05 *	2.52±0.22 **
	B.juncea	1.11±0.12	0.51±0.06	0.54±0.17	0.73±0.24	0.40±0.16	0.05±0.00	1.73±0.04
200	*B.napus*	1.34±0.17 **	0.44±0.03	0.59±0.07	0.30±0.04	0.40±0.04	0.07±0.01	1.35±0.09
	B.juncea	0.45±0.20	0.56±0.06 *	0.49±0.08	0.40±0.09	0.48±0.14	0.05±0.00	5.49±0.64 **

Data were the means±standard deviation (n = 4). Values followed by different asterisk indicate significant difference ($P \leq 0.05$) among different Cd levels in *B.napus* and *B.juncea* (*: $P < 0.05$, **: $P < 0.01$)

200 mg kg^{-1}, while the $BCF_{lower\ stem/soil}$, $BCF_{upper\ leaves/soil}$ were increased, the $BCF_{siliques/soil}$ had no differences. However, all the BCF showed significantly differences between the two Brassica species at 50 mg kg^{-1} Cd concentration condition ($p<0.05$), the BCF of *B. napus* was higher than that of *B. juncea*, expect for the $BCF_{lower\ stem/soil}$. The TF values of *B. napus* were 2.52 and 1.35 (≥1) under 50 and 200 mg kg^{-1} Cd treatments condition, respectively. The TF values of *B. juncea* were 1.73 and 5.49 (≥1) under 50 and 200 mg kg^{-1} Cd treatments condition, respectively (Table 1). The results revealed that the accumulation and transformation capacity pfCd in *B. napus* were higher than that in *B. juncea* at comparably lower (50 mg kg^{-1}) Cd stress condition.

The effect of Cd stress on mineral nutrients in the two brassica species

There were obvious differences between the two Brassica species for the six mineral nutrients in different parts of *B. napus* and *B. juncea* (Fig. 4). The Ca contents of *B. napus* were not affected by the Cd stress, only the Ca content was increased under 50 mg kg^{-1} Cd treatment condition. The Ca content in US of *B. juncea* was increased under 50 mg kg^{-1} Cd treatment condition, while in LL was decreased under 200 mg kg^{-1} Cd treatment condition (Fig. 4A). With the increase of Cd concentration, Mg content in LL, UL and S of *B. napus* was decreased. The Mg contents in LS, US, LL and UL of *B. juncea* were improved by the 50 mg kg^{-1} Cd treatment (Fig. 4B). The Cd stress had obviously effect on the Fe content in all parts of *B. napus* and *B. juncea*. The Fe content in roots of *B. napus* was increased under 50 mg kg^{-1} Cd treatment condition, which was 2.42 times higher than that in the normal condition. With the increase of Cd concentration, the content of Fe in LS, UL, LL and S of *B. napus* decreased, but the Fe content in UL was increased. The Fe contents in R, LS, UL and S of *B. juncea* were decreased with the increase of Cd concentrations. The Fe content in US of *B. juncea* was obviously increased under 200 mg kg^{-1} Cd treatment condition, while that in LL was also obviously increased at 50 mg kg^{-1} Cd concentration (Fig. 4C). The Zn contents in R, LS, LL and UL of *B. napus* were increased under 50 mg kg^{-1} Cd treatment, then decreased under 200 mg kg^{-1} Cd treatment, while the Zn content in S was decreased with the increase of the Cd concentrations. The Zn contents in LS, US, LL and UL of *B. juncea* were increased under 200 mg kg^{-1} Cd treatment

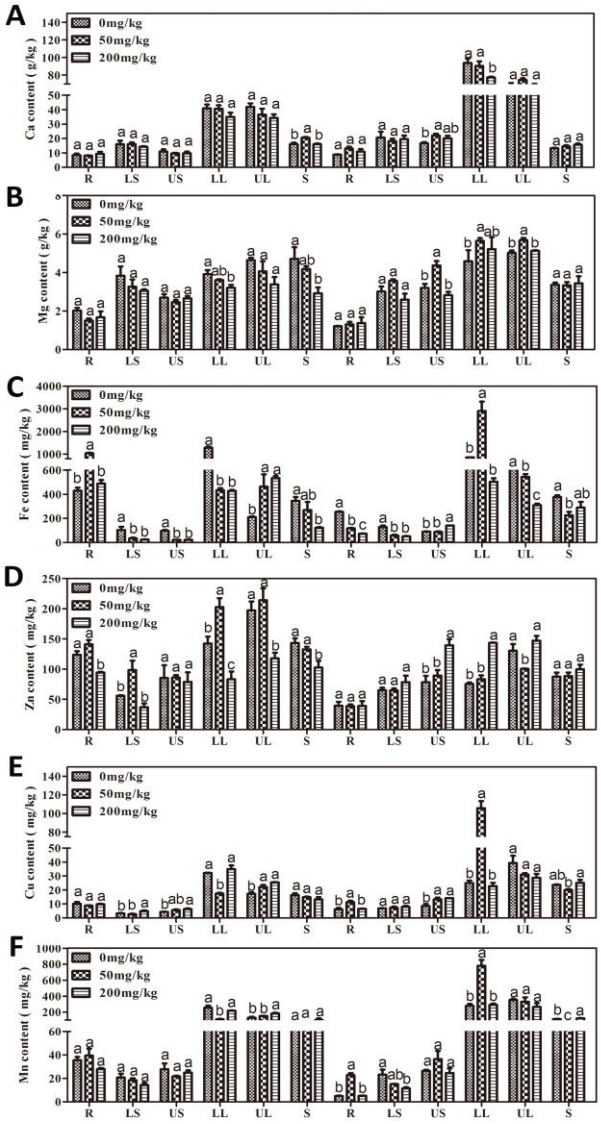

Fig. 4: The effect of Cd stress on Ca contents (A), Mg contents (B), Fe contents (C), Zn contents (D), Cu contents (E) and Mn contents (F) of different organs in the two Brassica species

Data were statistically evaluated using Duncan's test ($P < 0.05$). At least four biological replicates were performed for each treatment

condition, while other tissues of *B. juncea* were not affected by the Cd stress (Fig. 4D). The Cu contents in LS, US, LL and UL of *B. napus* were increased under 200 mg kg^{-1} Cd treatment condition, the Cu contents in R and S were no affected by the Cd treatments.

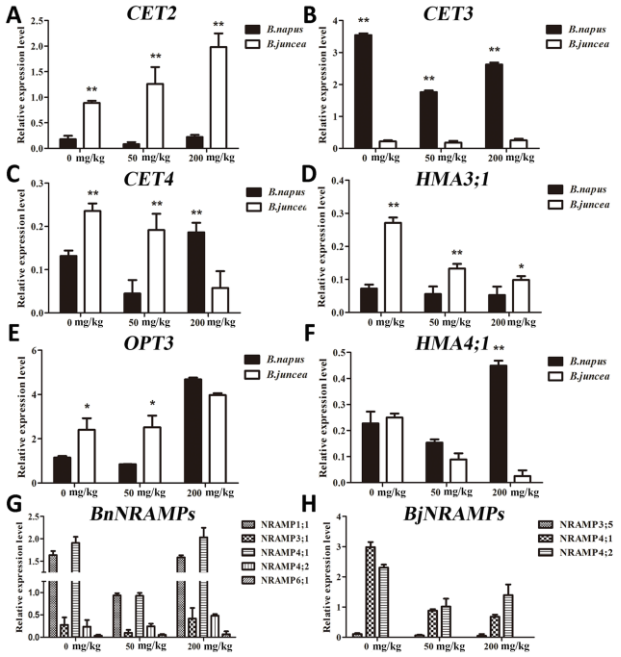

Fig. 5: The expression levels of CET2 (A), CET3 (B), CET4 (C), HMA3;1 (D), OPT3 (E), HMA4;1 (F) NRAMPs in *B. napus* (G) and *B. juncea* (H) in leaf of the two Brassica species in response to Cd stress

TData were statistically evaluated using Student's t test ($P < 0.05$). At least three biological replicates were performed for each treatment

The Cu contents in R, US, LL and S of *B. juncea* were affected by the Cd stress. The 50 mg kg^{-1} Cd treatment had improved the Cu contents in R and LL up to 11.20 and 105.81 mg kg^{-1} of *B. juncea*, which was 1.82 and 4.23 times higher than that in the normal condition (Fig. 4E). The Cd stress had significantly effect on the Mn contents in LL and UL of *B. napus*. The Mn contents in R, LS and US were weakly decreased with the increase of Cd concentrations of *B. napus*. The Mn contents in R, US and LL of *B. juncea* were increased under 50 mg kg^{-1} Cd treatment condition, while the Mn content in LS was decreased with the increase of Cd concentrations (Fig. 4F). The results indicated that the uptaken and translocation of micronutrients in the two Brassica species were affected in different levels under gradient increased Cd treatment. Interestingly, Zn contents were significantly affected in *B. napus*, which imply that Cd and Zn may compete the transport pathways in *B. napus*, which may not happen in *B. juncea*.

The effects of Cd stress on seeds characteristic of two brassica species

It can be seen from Table 2 that the fatty acid component of seeds were quite different under the Cd stress. In normal condition, the percentage of palmitic acid, stearic acid, oleic acid, linolenic acid and arachidonic acid were higher in seeds of *B. napus* than that of *B. juncea*, while the percentage of linolenic acid and erucic acid was lower in seeds of *B. napus*. Under 50 mg kg^{-1} Cd treatment, the percentage of palmitic acid, stearic acid, linoleic acid and arachidonic acid were obviously higher in seeds of *B. napus* than that of *B. juncea*, the percentage of oleic acid, linolenic acid and erucic acid were lower in seeds of *B. napus*. Under 200 mg kg^{-1} Cd treatment, the percentage of palmitic acid, linoleic acid and arachidonic acid were higher in seeds of *B. napus* (Table 2). The results suggested that the oil contents in both *B. napus* and *B. juncea* were almost = affected with = Cd treatment.

Gene expression analysis of Cd transport-related genes in leaves of two brassica species

In order to understand the relationship between Cd transport and the phenotypes of oilseed rape under Cd stress, 12 genes belonging to four gene families were selected. The 4 gene families were *CET*, *OPT*, *HMA* and *NRAMP*. It was shown that all the candidate genes from *B. napus* and *B. juncea* were significantly different in the expression levels at different Cd concentrations for 30 days exposure to Cd (Fig. 5). Compared with normal conditions, the expression levels of *CET2* gene in *B. napus* and *B. juncea* was up-regulated in Cd treatments, especially the expression level of *CET2* in *B. juncea* was up-regulated 2.23 times in 200 mg kg^{-1} Cd treatment condition (Fig. 5A). The expression levels of *CET3* gene was significant differences between *B. napus* and *B. juncea*. Compared with the normal condition, the expression levels of *CET3* gene in *B. napus* was down-regulated in the Cd treatments, then the expression levels of *CET3* gene in *B. juncea* was not affected by the Cd treatments (Fig. 5B). Under the 50 mg kg^{-1} Cd treatment condition, the expression levels of *CET4* gene from *B. napus* was down-regulated compared with the normal condition, then obvious up-regulated under the 200 mg kg^{-1} Cd treatment condition. The expression levels of *CET4* gene from *B. juncea* were down-regulated under the Cd treatments condition (Fig. 5C). The genes *HMA3;1* and *HMA4;1* in *B. napus* and *B. juncea* was lower expressed under the Cd treatments condition, while *HMA4;1* gene in *B. juncea* expressed highly when the Cd concentration was up to 200 mg kg^{-1} (Fig. 5D and F). The expression levels of *OPT3* gene from *B. napus* and *B. juncea* were up-regulated compared with the normal condition (Fig. 5E). Normally, the expression levels of *NRAMP* genes family were down-regulated under the 50 mg kg^{-1} Cd treatment compared with the normal condition. The *BnNRAMPs* were highly up-regulated under the 200 mg kg^{-1} Cd treatment condition. The Cd concentration up to 200 mg kg^{-1} didn't change the expression of *BjNRAMPs* (Fig. 5G and 5H). The result revealed that *BnCET4*, *BnOPT3*, *BnHMA4;1* in *B. napus*, and *BjCET2*, *BjOPT3* in *B. juncea* may play important roles in response to Cd stress.

Table 2: Effect of Cd stress on seeds fatty acid composition of *B.napus* and *B.juncea*

		0mg kg^{-1}		50 mg kg^{-1}		200 mg kg^{-1}	
		Brassica napus	*Brassica juncea*	*Brassica napus*	*Brassica juncea*	*Brassica napus*	*Brassica juncea*
Total fatty acid (%)	Palmitic acid C16:0	5.138±0.431***	2.530±0.185	3.906±0.211	2.772±0.775	3.916±0.019*	2.730±0.481
	Stearic acid C18:0	2.246±0.176**	1.517±0.083	1.718±0.264	1.588±0.234	1.827±0.139	1.960±0.228
	Oleic acid C18:1(9)	30.055±1.399	27.569±0.603	26.706±0.395	29.395±1.515	29.210±0.728	31.177±1.074
	Linoleic acid C18:2(9,12)	17.813±0.593***	7.994±0.379	15.536±0.243***	7.276±0.158	13.630±0.159**	8.248±1.538
	Linolenic acid C18:3(9,12,15)	4.444±0.029	5.134±0.116**	3.839±0.577	5.354±0.055*	3.349±0.144	4.019±0.375
	Arachidonic acid C20:1(11)	10.355±0.339*	8.682±0.984	9.473±0.586	9.291±0.854	9.901±0.164	9.686±0.150
	Erucic acid C22:1(13)	32.662±1.309	46.070±0.531***	38.426±0.667	45.736±0.963***	38.168±0.482	42.180±1.397*

Data were the means±standard deviation (n = 4). Means followed by different asterisk indicate significant difference (P≤0.05) among different Cd levels in *B. napus* and *B. juncea* (*: P < 0.05, **: P < 0.01, ***: *P*< 0.001)

Discussion

In soil, high Cd stress interferes with the physiological metabolism, inhibited the growth and development, reduced the biomass, and even lead to plant death. Some researchs have indicated that Cd can obviously inhibit plants growth (Li *et al.* 2013; Ehsan *et al.* 2014; Irfan *et al.* 2014; Hassan *et al*. 2016). In addition， the application of Cd had suppressed the Pn, Gs and Tr (Ehsan *et al.* 2014; Kaur *et al.* 2017). In this study, dry weights of roots, stems and leaves of the two Brassica species were reduced under Cd treatments, were consistent with that in *B .napus* L. (Ehsan *et al.* 2014). However, the biomass of both *B. napus* and *B. juncea* were not significant affected under the Cd stress. These results were similar to Wu's study (Wu *et al.* 2015). It is possible for plants to use the ability to accumulate Cd of their shoot to mitigate this danger in the soil (Rome *et al.* 2016). For example, *Thlaspi caerulescens* and *T. goesingense* can grow on the soil which containing Cd^{2+} or Ni^{2+} without any damage (Persans *et al*. 2001; Rigola *et al*. 2006). Furthermore, in the present study, according to the changes of biomass and the root/shoot ratio in response to Cd treatment, *B. juncea* was more sensitive to Cd treatment when comparing with *B. napus*.

Many studies have indicated that increasing Cd concentration can lead to the accumulation of Cd in plants (D'Alessandro *et al.* 2013; Wang *et al.* 2013; Pietrini *et al.* 2016). In this study, the Cd contents in roots, stem, leaves and seeds of the two Brassica species were significantly increased with the increasing of exogenous Cd concentration. While, *B. napus* accumulated a great deal of Cd in the shoots compared with *B. juncea*, which is in consistent with the study of Nouairi (Nouairi *et al.* 2006). Different plant varieties lead to significant differences in Cd uptake of *B. napus* and *B. juncea*. Generally speaking, the high Cd uptake capacity of *B. napus* may be related to its biomass and regulatory mechanisms (Fig. 1 and Table 1; Gallego *et al.* 2012). It is also meaningful to under the influence of cadmium on plants and the characteristics of cadmium accumulation in plants. It was reported that Cd in soil was absorbed by plant roots, most of which remain in roots, some were transported to shoots and then only a small was transferred to grains (Xue *et al.* 2013; Sterckeman *et al.* 2015; Wu *et al.* 2015). In the current study, *B. napus* can significantly accumulate more Cd in the shoot comparing with *B. juncea* under same Cd treatment conditions. It is likely that the difference of Cd accumulation between *B. napus* and *B. juncea* may be mainly affected by root-shoot process rather than soil-root process (Xue *et al.* 2013). Therefore, six Cd transport-related genes were expressed at different levels in both *B. napus* and *B. juncea* under Cd treatment. CET is one Cation-efflux transporters, which plays an important role in the detoxification of high concentration Cd. The expression levels of CET2 in *B. juncea* were higher than that in *B. napus*, which may be the reason why *B. juncea* accumulated less Cd. This may be due to reports that CET2 enhances heavy metal efflux in plants. Overexpression of BjCET2 in *B. juncea* increases the tolerance of heavy metal and accumulated a large number of Cd in leaves (Xu *et al.* 2009). The expression levels of CET3 in *B. napus* were higher than that in *B. juncea*. This may be due to the different role of BjCET3 under some stress conditions, which seems to be different from the cation-efflux transporters such as AtMTP1 and BjCET2 (Lang *et al.* 2011).

Additionally, OPT is another important oligopeptide transporter. In this study, Cd stress increased the expression of OPT3 gene to some extent under 200 mg kg^{-1} Cd treatment. Under high Cd treatment, plant tolerance to Cd increased, which increased the accumulation of Cd. This may be due to the significant expression of TcOPT3 on the ground. In addition, in situ hybridization analyses indicated that TcOPT3 is expressed in plant vascular systems, especially in the pericycle, which might be involved in the long-distance transport. When the expression of OPT3 is impaired, incorrect gene regulation mediates uptake and mobilization of trace metals, leading to excessive cadmium in seeds, not other metals (Hu *et al.* 2012; Mendoza-Cozatl *et al.* 2014). Recently, many studies have shown that the expression of HMA3 in the roots of *A. thaliana*, limiting the long-distance migration of Cd from root to shoot. So, the high expression of HMA3 in *B. juncea* limited the transport of Cd from root to shoot. But, over-expression of HMA3 is also responsible for high Cd accumulation and tolerance in other plants (Ueno *et al.* 2011; Zhang *et al.* 2016). In addition, the overexpression of AtHMA4 enhanced the transport of Cd and Zn from root to shoot, thus enhancing the tolerance of plant to the stress of heavy

metal (Verret *et al.* 2004; Wu *et al.* 2015). The combination of mutations in both homeologs of HMA4 was proposed as a strategy to limit the accumulation of Cd in leaves without affects the development (Liedschulte *et al.* 2017). In the present study, *B. juncea* demonstrated stronger ability to express HMA3;1, *B. napus* demonstrated stronger ability to express HMA4;1 under 200 mg kg^{-1} Cd treatment condition, especially for HMA4;1, leading to Cd accumulation in shoot increased.

NRAMP genes family plays a key role in absorption and transport of divalent transition metals. Researches have shown that the mutations selectively modifies Cd^{2+} and Zn^{2+} accumulation without affecting Fe^{2+} transport which was mediated by NRAMP4 in plants (Pottier *et al.* 2015). AtNRAMP6 is localized in a vesicular-shaped endomembrane and as a Cd intracellular transporter contributes to the detoxification of Cd (Cailliatte *et al.* 2009). Furthermore, OsNRAMP1 and OsNRAMP5 genes are expressed in roots have affinity with the cadmium, iron and manganese, participate in Cd absorption and transport (Ishimaru *et al.* 2012; Takahashi *et al.* 2014). The expression levels of *NRAMP* genes family in both *B. napus* and *B. juncea* were differ. The elevated expression of NRAMPs may also be a consequence rather than a cause of the increased Cd levels (Oomen *et al.* 2009). These results showed that genes related to Cd transport were significantly up-regulated or down-regulated under Cd treatment and interactived.

Conclusion

The *B. napus* cultivar used in the present study accumulated more Cd in the shoot compared to *B. juncea* under Cd treatment condition. According to the dry weight, biomass, root/shoot ration and photosynthesis related elements analysis, *B. napus* was more tolerant to Cd stress when comparing with *B. juncea*. The micronutrient detection analysis and the qRT-PCR assays revealed that there were different Cd uptake and translocation pathways in *B. napus* and *B. juncea* in response to Cd stress.

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