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# Selection of Mustard Oilseed Rape (*Brassica juncea L.*) for Phytoremediation of Cadmium Contaminated Soil

D. C. Su,[1] J. W. C. Wong[2]

[1] Department of Plant Nutrition, China Agricultural University, Beijing 100094, People's Republic of China
[2] Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, People's Republic of China

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The presence of heavy metals in agricultural ecosystems has raised concerns not only for crop quality but also for human health. Among heavy metals, cadmium is one of the major toxicological concerns (Shaw 1990). Special consideration should be paid to Cd pollution in soil-plant systems because of its high mobility and low toxic concentrations in organisms (Moreno-caselles et al. 2000). Agricultural soils can be contaminated with Cd from mining, smelting, industrial production and traffic, and also through utilization of sewage sludge, phosphate fertilizer and liming materials. About 14,000ha of agricultural soils are contaminated with Cd in China (Chen 1996). The remediation of such a large volume of heavy metal contaminated soils by conventional technologies previously developed for small heavily contaminated sites would be very expensive (Ebbs et al. 1997).

Phytoremediation has emerged as a potential cost-effective alternative to engineering-based technologies. Highly specialized plants that have evolved the ability to accumulate and tolerate very high concentrations of metals from soils may provide the basis for remediation of heavy metal contaminated sites. These plants were classified as hyperaccumulators by Brooks et al. (1977). Although metal hyperaccumulation is not a common phenomenon, some plant families, such as the Brassicaceae, are particulary well represented. *Thlaspi caerulescens* is the best known accumulator of Zn and Cd, and a common choice for phytoremediation (Baker 1987; Baker et al. 1994). The highest Cd and Zn concentrations observed without phytotoxic effect on plant growth were 1020 and 18455 mg $kg^{-1}$ for *Thlaspi caerulescens* (Brown et al. 1994). However, it has been conceded that this species may not be useful for phytoremediation because of its slow growth and poor biomass production (Black 1995; Brown et al. 1995a). To maximize the extent of phytoextraction, high biomass species with metal uptake characteristics similar to *Thlaspi caerulescens* will be required (Brown et al. 1995b). Kumar et al. (1995) reparted that several other members of the Brassicaceae from the genus *Brassica* may be heavy metal accumulators. Indian mustard is a high biomass and Cd accumulating crop within the Brassicaceae, which has been identified as a potentially useful plant species for phytoremediation (Salt et al. 1995). Hence this plant may be more useful than *Thlaspi caerulescens* for phytoremediation of Zn and Cd contaminated soil. However, this plant species has a limited geographical

*Correspondence to:* D. C. Su

range. Mustard oilseed rape (*Brassica juncea L.*) is one of the staple crops in China and is also a member of the Brassicaceae. China has a wide variety of oilseed rape varieties and it is possible that some genotypes may be Cd accumulators with potential for phytoremediation of Cd contaminated soil.

The objectives of the present study were therefore to select Cd toleranct and accumulating genotypes from 19 Chinese varieties of mustard oilseed rape and to compare the Cd phytoremediation potential of two selected mustard oilseed rape genotypes with that of Indian mustard.

## MATERIALS AND METHODS

Nineteen different varieties of mustard oilseed rape (*Brassica juncea L.*) obtained from different provinces of China and Indian mustard seeds obtained from the National Herbarium of the USA (accessions 426308) were sown in 9.5×12cm pots containing 750 g loamy soil of pH6.5 (1:5, $CaCl_2$). The soil was fertilized with N, P, K and spiked with Cd at a concentration of 20 mg $kg^{-1}$soil. All pots were arranged in a completely randomized design with three replications for each variety. After germination, the seedlings were thinned to 4 plants per pot and allowed to grow for 42 days, with pots being watered daily using deionized water. At the end of the growth period, plants were harvested by cutting the shoots at the soil surface. Shoots were washed with tap water and rinsed with double deionized water before oven dried at 70°C for 48 h, and the dry weight yields were measured. Oven-dried shoot tissues were ground using a stainless steel mill. Subsamples of ground materials were digested with a mixture of conc. $HNO_3$-$HClO_4$, and total Cd content were determined using Atomic Absorption Spectrometry (AAS). Standard reference materials of citrus leaves were digested and analysed for the purpose of quality control. The amounts of shoot Cd uptake by selected Cd tolerant genotypes of oilseed rape were calculated and compared with Indian mustard.

Two mustard oilseed rape (*Brassica juncea L.*) genotypes (Xikou huazi (X) and Zhucang huazi (Z) ) selected from the previous experiment, and Indian mustard (*Brassica juncea L.*) (I) were grown in 0.5 L tanks containing nutrient solution with different Cd concentration of 0, 5, 10 mg $L^{-1}$. The composition of the basal nutrient solution was as follows: 0.5 mM $K_2SO_4$; 0.25 mM $KH_2PO_4$; 0.325 mM MgSO4; 0.5 mM NaCl; 8 μM $H_3BO_3$; 1 μM $MnSO_4$; 0.4 μM $ZnSO_4$; 0.4 μM $CuSO_4$; 0.1 μM $Na_2MoO_4$; 4 μM Fe-EDDHA; 1 mM $CaSO_4$; 1 mM $NH_4NO_3$. The solution was buffered to pH 6.5 with either 0.1M HCl or 0.1M NaOH. Seeds of oilseed rape and Indian mustard were germinated and grown on vermiculite for 2 weeks and then for 4 weeks in the solution culture. There were four plants per tank and three replications for each treatment. The solutions were continuously aerated with an aquarium air pump and changed every 2 days. The set up was run under greenhouse conditions with temperature ranging from 20-30°C. Plants were harvested by cutting at the shoots base to separate the shoots and roots. Root volume and root length was measured with drain off water and square intercept method. Average radius of root was calculated based on root length and root volume. Shoot and root dry weight yields and Cd concentrations were measured using methods as delineated.

Seeds of the two selected mustard oilseed rape genotypes (Xikou huazi (X), Zhucang huazi (Z) ) and Indian mustard (I) were sown in 9.5×12cm pots containing 750 g loamy soil of pH 6.5. The soil was fertilized with 0.4 g N $kg^{-1}$ soil added in the form of $(NH_4)_2SO_4$, 0.4 g P and 0.4 g K $kg^{-1}$ soil added in the from of $KH_2PO_4$ and $K_2SO_4$. Treatments consisted of two oilseed rape genotypes and Indian mustard with 5 different Cd concentrations (0, 5, 10, 20 and 40 mg Cd $kg^{-1}$ soil) as $CdCl_2$. Treatments were arranged in a completely randomized design with three replications. After germination, the seedlings were thinned to 4 plants per pot and allowed to grow for 42 days, with pots being watered daily using deionized water. At the end of the growth period, plants were then harvested by cutting the shoots and roots at the soil surface and soil were removed from the roots gently. Shoot and root dry weight yields and Cd concentration were measured using methods described before.

All data were analyzed using a SAS statistical package through an IBM personal computer. One way ANOVA was carried out to compare the means of different treatments; where significant F values were obtained, differences between individual means were tested using the Least Significance difference Test.

## RESULTS AND DISCUSSION

The shoot Cd uptake of the 19 different varieties of oilseed rape from China and Indian mustard is given in Figure 1. Among the 19 varieties, only 5 genotypes of oilseed rape had higher Cd uptake than Indian mustard. One prerequisite for a plant to be used for phytoextraction Cd from Cd contaminated soil is its high ability in shoot uptake of Cd from soils. Amounts of shoot Cd uptake by different varieties of oilseed rape were calculated from shoot dry weight yield and shoot Cd concentration of oilseed rape, which can be used as an indicator of shoot Cd accumulation capability. There were no significant differences among these five varieties the oilseed rape and Indian mustard (Fig. 1). Number 13 (Xikou Huazi) and number 17 (Zhucang Hazi) had relatively higher shoot Cd uptake compared to Indian mustard (number 1). These two oilseed rape genotypes (Xikou Huazi and Zhucang Huazi) were selected for future experiments on phytoremediation of Cd contaminated soil and examination of their tolerance to Cd phytoxicity.

Table 1 shows the shoot and root dry weight yields of the two selected oilseed rape cultivars and Indian mustard grown in hydroponics at different concentrations of Cd. With an increase in the solution Cd concentrations, the shoot and root dry weight yields of the two selected oilseed rapes and Indian mustard decreased significantly. The shoot and root dry weight yields of the two selected oilseed rapes and Indian mustard did not differ significantly at each Cd concentration. This indicated that the two selected oilseed rape cultivars had the same Cd tolerance ability as Indian mustard. The root morphology characteristics under Cd stress could reflect the tolerance mechanism of the plant (Whiting et al. 2000). With an increase in Cd concentration in solution, the root volume of the oilseed rapes and

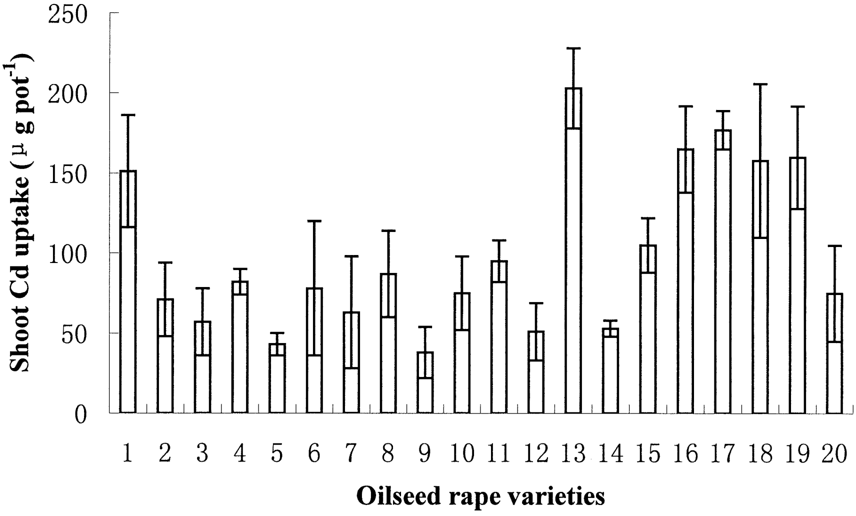


**Figure 1.** Shoot Cd uptake by 19 different varieties of mustard oilseed rape (*Brassica juncea L.*)(numbers 2 to 20) and Indian mustard (number 1) grown in soil artificially spiked with 20 mg $kg^{-1}$ Cd. (error bars are the standard deviations, n=3)

Indian mustard decreased significantly. Nevertheless, there was no significant difference between the two selected oilseed rape cultivars and Indian mustard. Root length showed similar trends to root volume. This indicated that Cd stress significantly reduced the root volume and length of the oilseed rape and Indian mustard. With an increase in Cd concentration, the average root radius decreased significantly accordingly. However, there were no significant difference between the oilseed rape and Indian mustard at each Cd concentration. Decrease in root elongation, root tip damage, and short and wide roots are the general symptoms of Cd stress plants (Punz and Sieghardt 1993). The roots of oilseed rape and Indian mustard became thinner when exposed to Cd stress (Table 1).

The shoot Cd concentrations of the two selected oilseed rape varieties and Indian mustard increased with an increase in solution Cd concentration. There were no significant differences among the three varieties at each particular Cd concentration.

Table 2 shows the shoot and root dry weight yields of the two varieties of oilseed rape and Indian mustard grown in soil spiked with different Cd concentrations. For soils spiked with 0 to 20 mg $kg^{-1}$ Cd, the dry weight yields of the two varieties of oilseed rape and Indian mustard did not show any significant difference. The shoot and root dry weight yields of the two species of oilseed rape and Indian mustard grown in soil spiked with 40 mg $kg^{-1}$ Cd were significantly lower than those grown in 0 to 20 mg $kg^{-1}$ Cd soil, indicating adverse growth effects at high Cd concentrations in soil. The oilseed rape species of Xikou Huazi had higher shoot

**Table 1.** Cadmium accumulation and susceptibility of the two selseted oilseed rape cultivars and Indian mustard grown in solution culture of different Cd concentration.

| Cd (mg $L^{-1}$) | Varieties of *Brassica juncea* | Shoot dry weight (g $pot^{-1}$) | Root dry weight (g $pot^{-1}$) | Root length (cm $pot^{-1}$) | Root volume ($cm^3$ $pot^{-1}$) | Average radius of root (mm) | Shoot Cd Conc. (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| 0 | I | 3.91±0.52 | 0.466±0. 116 | 531±11 | 4.07±0. 51 | 0.49±0. 03 | 0.71±0. 13* |
| | X | 4.05±0. 25 | 0.463±0. 041 | 524±26 | 4.37±0. 40 | 0.52±0. 02 | 0.51±0. 08* |
| | Z | 3.83±0. 03 | 0.549±0. 132 | 510±52 | 4.43±0. 93 | 0.53±0. 03 | 0.41±0. 11* |
| 5 | I | 1.45±0. 13* | 0.290±0. 021* | 388±31* | 3.03±0. 87* | 0.51±0. 03 | 934±88 |
| | X | 1.76±0. 34* | 0.277±0. 053* | 371±47* | 2.93±0. 40* | 0.50±0. 01 | 992±65 |
| | Z | 1.35±0. 46* | 0.219±0. 070* | 312±85* | 2.33±0. 61* | 0.49±0. 01 | 1055±108 |
| 10 | I | 0.41±0. 04 | 0.043±0. 012 | 126±21 | 0.35±0. 05 | 0.30±0. 01* | 1063±85 |
| | X | 0.57±0. 17 | 0.076±0. 010 | 176±12 | 0.77±0. 06 | 0.37±0. 03* | 993±26 |
| | Z | 0.48±0. 06 | 0.071±0. 021 | 155±20 | 0.67±0. 15 | 0.37±0. 02* | 966±25 |

Values are Means±SD (n=3).

* Significantly different from the other two Cd concentration treatments at $p<0.05$ according to LSD-test. Comparisons are among the same varieties.

I- Indian mustard; X- oilseed rape of Xikou Huazi; Z- oilseed rape of Zhucang Huazi.

**Table 2.** Cd uptake and shoot removal rate by selected oilseed rape cultivars grown in a loamy soil spiked with different concentration of Cd.

| Soil Cd (mg $kg^{-1}$) | Varieties of *Brassica juncea* | Shoot dry weight (g $pot^{-1}$) | Shoot Cd Conc. (mg $kg^{-1}$) | Root dry weight (g $pot^{-1}$) | Root Cd Conc. (mg $kg^{-1}$) | Shoot Cd uptake in total plant (%) | Soil Cd removal rate by shoot (%) |
|---|---|---|---|---|---|---|---|
| 0 | I | 3.00±0.50 | 0.83±0.29 | 0.35±0.10 | 0.83±0.09 | 92 | -- |
| | X | 2.98±0.70 | 0.83±0.26 | 0.27±0.11 | 0.63±0.06 | 95 | -- |
| | Z | 3.35±0.69 | 0.67±0.29 | 0.24±0.08 | 0.63±0.07 | 96 | -- |
| 5 | I | 3.23±0.49 | 33.0±4.2 | 0.40±0.03 | 40.1±3.1 | 87 | 2.7 |
| | X | 4.16±0.16 | 31.8±1.8 | 0.38±0.09 | 31.1±3.9 | 92 | 3.5 |
| | Z | 2.42±0.27 | 45.5±8.5 | 0.18±0.05 | 31.4±3.7 | 94 | 2.6 |
| 10 | I | 3.06±0.51 | 64.2±6.0 | 0.26±0.07 | 91.5±11.3 | 89 | 2.6 |
| | X | 3.95±0.74 | 67.2±12.0 | 0.31±0.05 | 78.8±11.1 | 92 | 3.5 |
| | Z | 3.36±0.76 | 76.3±14.2 | 0.22±0.06 | 77.9±7.1 | 94 | 3.3 |
| 20 | I | 2.23±0.63 | 182±23* | 0.21±0.09 | 268±28* | 88 | 2.6 |
| | X | 4.58±0.29** | 127±12* | 0.39±0.12** | 204±38* | 88 | 3.9 |
| | Z | 1.94±0.81 | 198±18* | 0.13±0.08 | 208±31* | 94 | 2.5 |
| 40 | I | 0.71±0.01* | 315±25* | 0.07±0.01* | 342±42* | 90 | 0.7 |
| | X | 0.34±0.11* | 306±43* | 0.03±0.01* | 252±24* | 94 | 0.4 |
| | Z | 0.44±0.12* | 352±39* | 0.03±0.02* | 272±41* | 95 | 0.5 |

Values are Means ±SD (n=3).

* Significantly different from the other Cd concentration treatments at $p<0.05$ according to LSD-test. Comparisons are among the same varieties.

** Significantly different from Indian mustard and Zhucang Huazi at $p<0.05$ according to LSD-test. Comparisons are among the same soil Cd concentration.

and root biomass production than Zhucang Huazi and Indian mustard at a Cd concentration of 5 to 20 mg kg soil, especially at a soil Cd concentration of 20 mg $kg^{-1}$. This indicated that Xikou Huazi is likely to have a higher Cd tolerance ability than Indian mustard and another oilseed rape (Zhucang Huazi). It appears that at a high soil Cd concentration of 40 mg $kg^{-1}$ both the oilseed rapes and Indian mustard could not achieve a good growth performance, with significantly lower shoot and root biomass, stunted and chlorotic growth which was consistent with the symptoms of Cd toxicity.

Table 2 also gives the shoot and root Cd concentrations of the two selected oilseed rape cultivars and Indian mustard. With an increase in Cd concentration in the soil from 0 to 40 mg $kg^{-1}$, the shoot and root Cd concentration increased in almost a linear relationship. The Cd concentration in shoots and roots of the two oilseed rape cultivars and Indian mustard did not differ significantly at each initial soil Cd concentration. The Cd concentration in the shoots of the two oilseed rape cultivars and Indian mustard ranged from 306 to 352mg $kg^{-1}$ and 252 to 342mg $kg^{-1}$ in the roots when soil Cd concentration was 40 mg $kg^{-1}$. Phytoextraction of contaminants depends on shoot biomass production and Cd concentration in the shoots (Ebbs et al. 1997; Ebbs and Kochian 1998). Compared with the recognized hyperaccumulator Indian mustard, the oilseed rape variety Xikou Huazi had a similar or even better Cd extraction capacity from Cd contaminated soil. It can be estimated that 88-96% of the Cd uptake by the oilseed rape and Indian mustard accumulated in the shoots at each level of soil Cd addition.

Shoot Cd removal rate is a comprehensive index for determining the ability of a plant species to phytoremediate Cd contaminated soil. It depends on shoot dry weight yield and shoot Cd concentration. Table 2 shows that Xikou Huazi cultivar of oilseed rape had a similar or even better Cd removal rate as compared with Indian mustard and cultivar Zhucang Huazi at a soil Cd concentration range of 5 to 20 mg $kg^{-1}$. The shoot Cd removal percentage of Xikou Huazi was 3.5% to 3.9% under the present experimental conditions with a growth period of 42 days, while Indian mustard and Zhucang Huazi were lower than 2.7%. Shoot Cd removal rate indicated that Xikou Huazi cultivar of oilseed rape demonstrated a higher phytoremediation potential for Cd contaminated soil.

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## REFERENCES

Baker AJM (1987) Accumulators and excluders – Strategies in the response of plants to heavy metals. J Plant Nutr 3:643-654

Baker AJM, Reeves RD, Hajar ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J.& C. Presl (Brassicaceae). New Phytol 127:61-68

Black H (1995) Absorbing possibilities: phytoremediation. Environ Health Perspect 103:1106-1108

Brooks RR, Lee J, Reeves RD, Jaffre T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J Geochem Explor 7:49-58

Brown SL, Chaney RL, Angle JS, Baker AJM (1994) Phytoremediation potential of *Thlaspi caerulescens* and Bladder compion for Zinc and Cadmium-contaminated soil. J Environ Qual 23:1151-1157

Brown SL, Chaney RL, Angle JS, Baker AJM (1995a) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils. Environ Sci Technol 29:1581-1585

Brown SL, Chaney RL, Angle JS, Baker AJM (1995b) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. J Soil Sci Soc Am 59:125-133

Chen HM (1996) Heavy metals in plant – soil systems. Science press, Beijing

Ebbs SD, Lasat MM, Brady DJ, Cornish J, Gordon R, Kochian LV (1997) Phytoextration of cadmium and Zinc from a contaminated soil. J Environ Qual 26:1424-1430

Ebbs SD, Kochian LV (1998) Phytoextraction of Zinc by oat (*Avena sativa*), barley (*Hordeum vulgare*), and Indian mustard (*Brassica juncea*). Environ Sci Technol 32:802-806

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: The use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232-1238

Moreno-Caselles J, Moral R, Perez-Espinosa A, Perez-Murcia MD (2000) Cadmium accumulation and distribution in cucumber plant. J Plant Nutr 23:243-250

Punz WF, Sieghardt H (1993) The response of roots of herbaceous plant sepcies to heavy metals. Environ Exp Bot 33:42-47

Salt DE, Prince RC, Pickering IJ, Raskin I. (1995) Mechanisms of cadmium mobility and accumulation in Indian Mustard. Plant Physiol 109:1427-1433

Shaw AJ (1990) Heavy metal tolerance in plants: Evolutionary aspects. CRC Press, Boca Roton, FL

Whiting SN, Leake JR, McGrath SP, Baker AJM (2000) Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. New Phytol 145:199-210