Journal of Radioanalytical and Nuclear Chemistry, Vol. 264, No. 2 (2005) 303–306

^{109}Cd uptake and translocation in a soybean plant under different pH conditions

T. Ohya,* H. Iikura, K. Tanoi, H. Nishiyama, T. M. Nakanishi

Graduate School of Agricultural and Life Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

(Received April 6, 2004)

Though it has been reported that the accumulation of Cd in plant tissue increases with the decrease of the pH in soil, the mechanism of Cd accumulation in plant has not yet been clarified. Therefore, we investigated the effect of rhizosphere pH condition on the Cd accumulation in a soybean plant root, which is a gate for Cd uptake, using ^{109}Cd tracer and an imaging plate (IP). Cadmium uptake by root tissue was found to be a fast reaction, since the amount of Cd uptake reached the plateau within about 2 hours (its time constant was about 20 minutes), Cd was easily transported into root apoplast, and moreover, its dynamics did not depend on an environmental pH condition (pH 4.5, 6.5). However, it was suggested that the amount of Cd taken up from the root was much higher in acidic conditions. Through image analysis taken by the IP, the accumulation and translocation of Cd were studied in roots.

Introduction

Essential transition metals for plant growth such as Fe, Zn, and Mn are contained in the soil, and also transition metals which are not required, such as Cd, Pb, Hg etc., can cause various metabolic disorders in plants[1] when their concentration in tissue is over a certain threshold. Therefore, it is a necessary function of plants to suppress the uptake or translocation of toxic metals to the main organs in order to acclimatize with various soil environments. The investigation of the plant strategic mechanism for heavy metals in soil has been receiving attention in order to secure safe food and environmental clean-up, because the soil contamination is a serious world-wide problem with industrial development. In this study we focused on Cd toxicity, and studied the effect of rhizosphere pH condition on the Cd uptake and accumulation in a soybean plant.

To reduce Cd toxicity to the crop plant, increasing the pH value of the soil has been known as an effective method. For instance, the relation between pH condition in soil and the Cd accumulation in wheat and soybean plants were reported.[2–4] However, it was difficult to estimate the effect of pH difference on Cd toxicity in detail from these results, because there were many different factors, such as variation of cultivar or components of mineral and organic materials included in soil. Therefore, we employed a simple condition for a plant sample to study the physiological reaction for Cd under different pH values. To study Cd behavior, ^{109}Cd tracer was used and through radiography using an IP, with a resolution of 25 μm, adsorption, absorption and the manner of translocation of Cd were analyzed.

* E-mail: atoha@mail.ecc.u-tokyo.ac.jp

Experimental

Sample preparation

Soybean seedlings (*Glycine max* (L.) Merr.) were grown in vermiculite for 3 days to germinate in planter, which was covered with aluminum foil (temperature $T = 27$ °C, relative humidity RH=free, in dark room), followed by culturing in a 0.2 mM calcium chloride solution with aeration for one day ($T = 25$ °C, RH=free, about 50–60%). Then, similar size of seedlings were selected measuring their cotyledon (41.0±9.9 mm) and root length (63±12 mm) to minimize the standard deviation among the samples.

Cd adsorption

The selected soybean seedlings were treated in calcium chloride solution (soln. A), of which the pH was adjusted to 4.5 for 3 hours. In addition, similar operation was performed at pH 6.5 condition. Then, they were soaked in Cd solution (soln. B*) for 10 seconds. After washing three times with the corresponding solutions,* C, D and E for 10 seconds, and wiping with soft paper, 3 cm of the root tip was cut and exposed to an IP in a freezer to avoid Cd movement in tissue. To examine the Cd absorption into the inner part of the root tissue, roots were sliced to a few tens of micrometers, in thickness, in a cryostat (MICROM HM505N: Zeiss Co.), fixed on a slide glass, and exposed to an IP in a shielded box under room temperature. The image in the IP (BAS-IP MS 3543: Fujifilm) was scanned by a high performance image analyzer (FLA5000: Fujifilm Co. highest

* soln. A: 0.2 mM $CaCl_2$, 20 mM MES/NaOH; soln. B: (soln. A) + 1 μM $CdCl_2$; soln. B*: (soln. B) +0.11 nM ^{109}Cd (0.325 kBq/ml: Amersham Co.); soln. C: (soln. A)+ 10 μM $CdCl_2$; soln. D: (soln. A) + 20 mM EDTA. The pH of each solution was adjusted using a pH meter (HM-30S: DKK-TOA Co.).

0236–5731/USD 20.00

Akadémiai Kiadó, Budapest
Springer, Dordrecht

resolution = 25 μm) with 16 bit data size. The region of interest (ROI) was set in the range of PSL (photo stimulated luminescence) value (about 30,000–65,000 counts), where the linear relation between the PSL and ^{109}Cd concentration was shown. In the case of sliced tissue image, quantitative analysis based on PSL was not performed because the value was very low.

Short term Cd uptake

Soybean seedlings after pretreatment were soaked in solution A for 3 hours, with pH was adjusted to 4.5 (or 6.5). Then, they were cultured in soln. B* from 10 to 210 minutes for cadmium uptake from root tissue. The sample was washed three times with solution B for ten seconds to remove Cd adsorbed on root surface. After washing, each root part was cut and exposed to an IP and analyzed in the same manner as written in the solution on 'Cd adsorption'.

Cd translocation

Soybean seedlings after pretreatment were grown in Cd solution (solution B) for 4 days, after which pH was adjusted to 4.5 (or 6.5) for 4 days. On the 5th day (= 9 days from the sowing), the culture solution was exchanged from B to B* for only 2 hours (pulse treatment of ^{109}Cd). Then their culture solution was exchanged again from solution B^+ to solution B after washing three times with solution D for 10 seconds, and they continued to grow. The light was set day : night = 12 : 12, and Cd treatment was performed in the light phase. The culture solution was renewed every day to keep the same pH and Cd concentration. Then plant samples were harvested and separated into 4 parts; EP: epicotyl, UH: upper part of the hypocotyl, the ground part of the stem with red color, LP: lower part of the hypocotyl, the upper part of the root without developing lateral roots, R: main part of the root. Each part was exposed to an IP and analyzed in the same manner described above. Similarly, the sliced tissue, 50 μm in thickness, was also exposed to an IP.

Results

Figure 1 shows the Cd adsorption amount on root for short term treatment of 10 seconds. The vertical axis indicates the normalized PSL, where the standard PSL was that measured at the center of the root without washing.

The decrease of PSL was in the order of 10% $CaCl_2$, 10 μM Cd and EDTA. Since the normalized PSL corresponds to Cd concentration, 10 to 20% of the adsorbed amount of Cd was removed by washing with $CaCl_2$ or Cd solution. When EDTA solution was applied, almost all of the Cd was washed out from the root, suggesting that Cd adhered only weakly at the outer part of the root by short term treatment.

In the case of the radiograph, the outline image of the root disappeared after washing with EDTA.

Figure 2 shows the amount of ^{109}Cd taken up by the root for a relatively short term, within 210 minutes. Cadmium absorption was started as soon as the root was soaked in solution B, and reached a plateau after about 100 minutes, and increased afterwards.

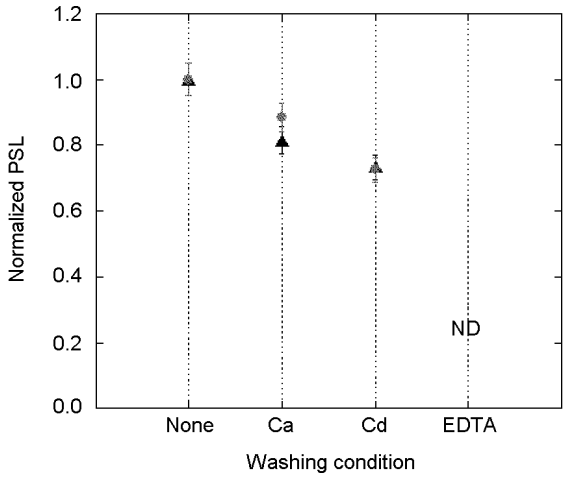

Fig. 1. The effect of pH difference for Cd adsorption on root surface. The roots of the seedlings were cultured in a ^{109}Cd solution, whose pH was adjusted at 4.5 (the other was at pH 6.5), only for 10 seconds. They were excised and exposed to an IP after washing in each condition (horizontal axis). Symbol indicate the pH conditions: ▲ pH 4.5, ● pH 6.5 data points and error bars represent means (n = 4) and SE. The experiment was repeated two times

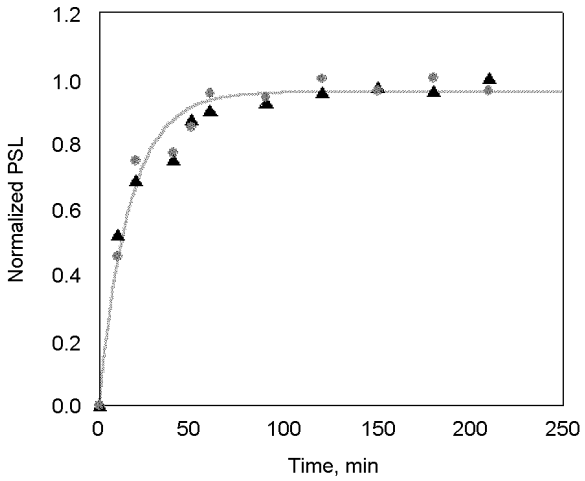

Fig. 2. Cd uptake in root tissue for a short term. Each data point corresponds to the different sample. The solid line indicates a fitting curve at pH 6.5. The experiment was repeated two times; ▲ pH 4.5, ● pH 6.5

The time constant for this absorption was about 20 minutes, and the value was almost the same between the plants treated with pH 4.5 and pH 6.5. From the IP image, the accumulation of Cd was especially high at root tips, whereas the root diameter was almost constant within 3 cm from the root tip (data not shown). Furthermore, the radiograph of the sliced root treated with Cd solution for 30 minutes showed that Cd was penetrated as far as the center tissue of the root.

Soybeans were pretreated with Cd solution (soln. B), and the fresh weight of each part (EP, UH, LH, R) was measured for about ten days. There was no significant difference among the weight of EP, UH and LH, between pH 4.5 and pH 6.5 for 10 days.

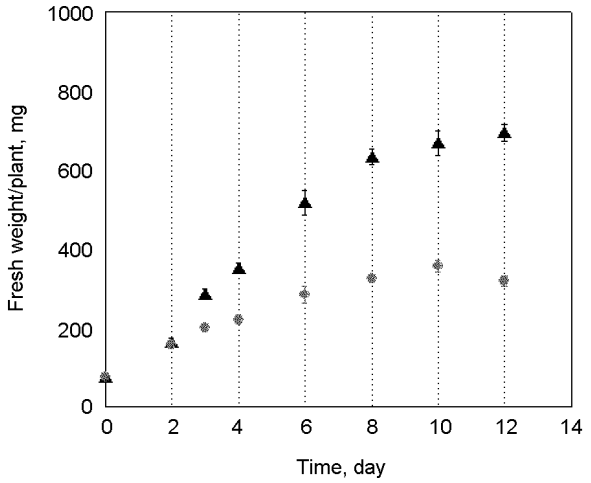

Fig. 3. Root growth curve treated with Cd at pH 4.5 (▲) and pH. 6.5 (●). Seedlings were grown for 2 weeks in a cadmium solution corresponding pH value. Data points and error bars represent means (n = 8) and SE

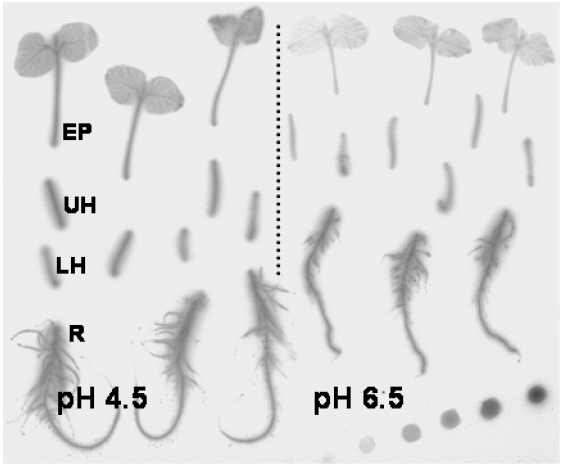

Fig. 4. Radiographic image of soybean seedlings, 2 days after ^{109}Cd treatment. The grayscale corresponded to the PSL. The darker parts in the image indicate higher amount of radioactivity. Dotted line divide the samples under different pH condition

However, the root weight at pH 6.5 was drastically decreased to half of that at pH 4.5 (Fig. 3), whereas the root at pH. 4.5 continued to grow afterwards. The main morphological changes observed at pH 6.5 were: (1) root changed color, in stripe, from white to dark brown, (2) lateral root growth was especially inhibited, and (3) root hardened. These clear changes appeared after three days of Cd treatment. Along with the morphological differences of the root, the length of the stem (UH) was not increased after 4 days, therefore, pulse treatment of ^{109}Cd was performed for 2 hours at this stage to study translocation of Cd within a plant.

Figure 4 shows the radiographic image at 2 days after pulse treatment of ^{109}Cd. The darker part in the image indicates higher amount of radioactivity. The overall PSL value at pH 4.5 was higher than that at pH 6.5 and the UH part showed high PSL, next to the R part. In the EP part, stem and node showed high PSL. In the case of leaf, vein and region near the vein showed high PSL. On the other hand, the root treated at pH 6.5 showed that PSL was as high as that at pH 4.5, and especially, the main root showed high Cd accumulation, while PSL of the other parts were low. The PSL in EP was the threshold defined for this analysis, that is, the linear relation between the radioactivity and PSL value was not shown in this region.

The radiographic image of sliced root showed that parenchyma tissue showed higher PSL value, rather than vascular bundle, and the discoloration part at pH 6.5 also showed high PSL (Fig. 5a). The IP image of UH and EP parts showed that, at pH 4.5, PSL was higher in the following order: epidermis ≥ vascular bundle > parenchyma, and PSL of central part was very low (Fig. 5b), whereas, at pH 6.5 overall PSL distribution in these parts was similar to that at pH 4.5 with low PSL value. However, we could not distinguish the Cd in the vessel from that in phloem.

Discussion

Figure 1 shows Cd uptake for a few hours from root tissue. Cd uptake was very fast, followed by reaching a stable state at about 100 minutes later. Here, the high Cd accumulation near the root tip is most likely attributed to the adsorption to mucilage at root surface,[5] because Cd accumulation could not be recognized near the root tip from the sliced root image in IP.

With respect to Cd uptake findings, CATALDO[6] has reported that the reaction is dominated by the physical effect (diffusion) and not so much dependent on the metabolic one, in higher Cd concentration (>0.5 μM). CUTLER[7] has observed in barley excitation root that most of the Cd (about 75%) taken up could be desorbed only by soaking the root in calcium solution for 2 hours.

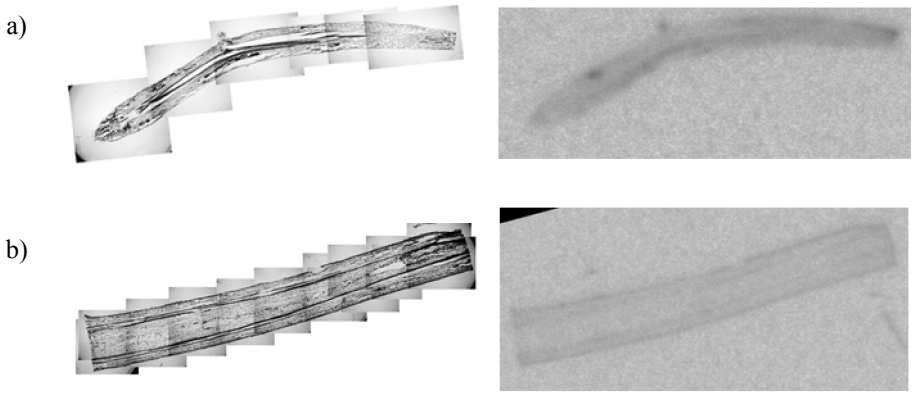

Fig. 5. Microscopic image (left side) and radiographic image (right side) of root at pH 6.5 (a), and stem at pH 4.5 (b). The grayscale corresponded to the PSL. The darker parts in the image indicate higher amount of radioactivity

Therefore, it was suggested that the reason why Cd could not be desorbed completely by washing with the EDTA solution in this report, was that EDTA could not diffuse further into the tissue within 10 seconds. Cadmium absorbed from short term treatment may exist in the apoplast (free space) rather than in the symplast of the root, which suggestion was not contradictory to the results that most of the Cd was located in the cell wall than the cytoplasm of the *Polygonum cuspidatum* root.[8]

Figure 4 shows radiographic image of the soybean on the 2nd day after pulse treatment of ^{109}Cd. Cadmium was localized in tissue, i.e., at root, stem, node, vein and neighboring part. It was suggested that Cd translocation was mainly performed via vascular bundle. The same results were reported for potato,[9] wheat[10] and rice.[11] In the root, some cells near the epidermis changed color to brown at pH 6.5, which part corresponded to the Cd localized site. Thus, at pH 6.5, Cd translocation from root to shoot might be suppressed by compartmentalized Cd complex.[12]

Cadmium translocation after 4 days of the treatment was largely dependent on pH value, that is, the activity of Cd translocation and absorption into stem tissue at pH 4.5 was much greater than that at pH 6.5.

In conclusion, it showed that Cd could infiltrate into root tissue easily in spite of the pH condition of culture solutions. On the other hand, the amount of Cd transelocated from root to shoot was highly dependent on pH (pH 4.5 > pH 6.5), suggesting an important role of the root for Cd toxicity.

References

1. J. BARCELÓ, CH. POSCHENRIEDER, J. Plant Nutr., 13 (1990) 1.
2. R. G. GARRET, A. I. MACLAURIN, E. J. GAWALKO, R. TKACHUK, E. M. GWENDY, HALL J. Geochem. Expl., 64 (1998) 101.
3. D. P. OLIVER, K. G. TILLER, A. M. ALSTON, G. D. COZENS, R. H. MERRY, Aust. J. Soil Res., 36 (1998) 571.
4. S. N. IRETSKAYA, S. H. CHIEN, Commun., Soil Sci. Plant Anal., 30 (1999) 441.
5. J. A. C. VERKLIEJ, H. SCHAT, Evolutionary Aspects of Heavy Metal Tolerance in Plant, CRC Press, Boca Raton FL, 1990, p. 179.
6. D. A. CATALDO, T. R. GARLAND, R. E. WILDUNG, Plant Physiol., 73 (1983) 844.
7. J. M. CUTLER, D. W. RAINS, Plant Physiol., 54 (1974) 67.
8. H. NISHIZONO, K. KUBOTA, S. SUZUKI, F. ISHII, Plant Cell Physiol., 30 (1989) 595.
9. R. J. REID, K. R. DUNBAR, M. J. MCLAUGHLIN, Plant Cell Environ., 26 (2003) 201.
10. R. M. WELCH, J. J. HART, W. A. NORVELL, L. A. SULLIVAN, L. V. KOCHIAN, Plant Soil, 208 (1999) 243.
11. M. CHINO, J. Soil Manure Japan, 44 (1973) 204 (in Japanese).
12. D. E. SALT, G. J. WAGNER, J. Biol. Chem., 268 (1993) 12297.