Plant and Soil 100, 21–34 (1987). Ms. 100-04

Effect of calcium on the absorption and translocation of heavy metals in excised barley roots: Multi-compartment transport box experiment

T. KAWASAKI and M. MORITSUGU
Institute for Agricultural and Biological Sciences, Okayama University, Kurashiki 710, Japan

Key words Absorption Barley Cd Excised roots Mn Multi-compartment transport box Radioisotope Translocation Zn

Summary The effect of Ca on the absorption and translocation of Mn, Zn and Cd in excised barley roots was studied using a multi-compartment transport box technique. A radioisotope (^{54}Mn, ^{65}Zn or ^{115m}Cd)-labelled test solution was supplied to the apexes of excised roots and the distribution pattern in the roots was examined in the absence or presence of Ca. Results obtained were as follows.

Addition of Ca to the test solution reduced the absorption of Mn and inhibited drastically its translocation in excised roots. With increasing concentrations of Ca in test solutions, its inhibitory effects on the absorption and translocation of Mn became severe.

Similar results were observed for the absorption and translocation of Zn. Ca in the test solution decreased the absorption and inhibited drastically the translocation of Zn; as in the case of Mn, higher concentrations of Ca had severe effects on these functions.

It was also evident that the addition of Ca to the test solution reduced the absorption of Cd at all levels of Cd concentration (1, 10, and 100 μM). Cd absorption decreased with increasing concentrations of Ca in the test solution. However, Ca accelerated the translocation of Cd in excised roots supplied with test solutions containing up to 10 μM Cd. At 100 μM Cd, addition of Ca caused a negligibly small acceleration of Cd translocation.

The accelerating effect of Ca on Cd translocation, especially "xylem exudation", decreased markedly with the addition of 2,4-dinitrophenol, but not with the addition of chloramphenicol or p-chloromercuribenzene sulphonic acid. When barley plants were supplied with only $CaSO_4$ during the entire growing period, that is, plants were not supplied with nutrient solution on the last day of this period, Ca had no accelerating effect on Cd translocation in excised roots.

Introduction

Mn and Zn have been established to be essential microelements for higher plants, while their excesses give significant injurious effects on plant growth[20]. Numerous investigations have been carried out on the absorption of Mn[19,21,23,24] and Zn[1,2,4,5,6,26,27] by plant roots. Several researchers have also reported on microelement transport from roots to shoots[4,10,25]. However, there have been no reports about the translocation of Mn and Zn in plant roots.

On the other hand, it is well known that Cd is not only harmful to humans[18], but also toxic to plants[20]. Though many researchers have been carried out on the absorption of Cd by plants[8,9,11,12,13,22], there has been also no report thus far dealing with the transport pattern of Cd in plant roots.

To investigate in more detail the absorption and translocation of heavy metals, the distributions of Mn, Zn and Cd in plant roots were examined in relation to Ca supply in the present experiment, using a multi-compartment transport box[16,17].

Materials and methods

Excised roots of 4-day-old barley plants (*Hordeum vulgare* L., cv. Akashinriki) were used as experimental materials in this investigation. Seeds of barley were allowed to germinate for 24 h in aerated water at 25°C. The germinating seeds were spread over a layer of plastic screen, and grown with 0.25 m*M* $CaSO_4$ for 48 h. Thereafter, a nutrient solution was supplied for 24 h in the dark at 25°C under continuous aeration. Composition of the nutrient solution used was as follows: KNO_3 4.0 m*M*, $NH_4H_2PO_4$ 1.0 m*M*, $CaCl_2$ 1.0 m*M*, $MgSO_4$ 1.0 m*M*, Fe 1.0 ppm (as Fe-citrate), B 0.5 ppm (as H_3BO_3), Mn 0.5 ppm (as $MnCl_2$), Zn 0.05 ppm (as $ZnSO_4$), Cu 0.02 ppm (as $CuSO_4$) and Mo 0.01 ppm (as $(NH_4)_6Mo_7O_{24}$). The roots of the seedlings were excised, washed thoroughly with deionized water and used for transport experiments.

In the present investigation, a multi-compartment transport box, the description of which was given in detail in the previous papers[16,17], was used. As can be seen in Fig. 1, the apparatus consists of 4 compartments, each of which is about 10 mm long, 50 mm wide and 15 mm deep, with plexiglass barriers between the compartments. Roman numerals in the figure indicate the position of each compartment.

Excised barley roots were set horizontally so that the apical part of the root was put in compartment I and the basal cut end in compartment IV (see Fig. 1). Then the upper half of each barrier was put on the roots without crushing them. The barriers were sealed with vaseline to prevent leakage of the test solution. In all of the experiments, 8 roots were used for each treatment.

^{54}Mn, ^{65}Zn and ^{115m}Cd were used to label manganese, zinc and cadmium, respectively, in the test solutions. Compartment I was supplied with a radioisotope-labelled test solution, and compartments II, III and IV with a non-labelled test solution having the same chemical composition as that of the radioisotope-labelled one. In all treatments, sodium tartrate was added at 1.0 m*M* concentration to avoid pH changes. The pH of all the test solutions was checked and adjusted to 5.0. The absorption treatment was continued for about 20 h at 25°C in the dark.

After the absorption period, solutions in each compartment were placed in sample tubes. The roots were cut at the barriers between each compartment and each segment was put into a separate sample tube. Only the apexes of roots in compartment I, which were supplied with a radioisotope-labelled test solution, were sampled after about 5 min of desorption treatment with non-labelled

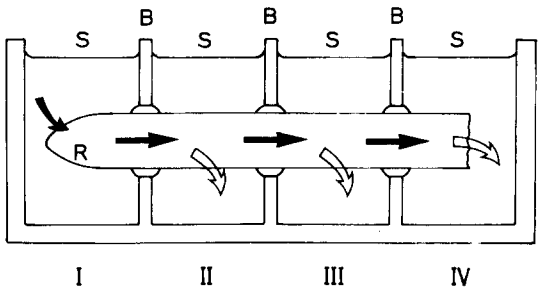

Fig. 1. Multi-compartment transport box. *Black arrows* show diagrammatically the absorption and translocation of ions in a plant root, and *white arrows* show the efflux of ions from a plant root. **I, II, III** and **IV**: the position of each compartment; **R**: root; **S**: test solution; **B**: plexiglass barrier. For a further description, see Materials and methods.

solutions having the same chemical composition as that of the radioisotope-labelled one. Radioactivity of the samples was measured with a well-type scintillation counter for ^{54}Mn and ^{65}Zn, and by means of Cerenkov radiation[3] with a liquid scintillation counter for ^{115m}Cd. The amounts of Mn, Zn and Cd in each part of the excised roots and in the solution of each compartment were calculated, based on the radioactivities of ^{54}Mn, ^{65}Zn and ^{115m}Cd, respectively. Results of each treatment are shown as the mean values of three to eight replicates, and the results are expressed in μmol/g fresh weight of roots/24 h. These results show the distribution of Mn, Zn and Cd through the roots, and the sum of these results gives total amounts of Mn, Zn and Cd absorbed in the apex of the roots.

Results

Mn absorption and translocation in excised roots

The absorption and distribution of Mn in excised barley roots were examined using various concentrations of Mn in the absence or presence of Ca (500 μM). The results for 1, 10 and 100 μM treatments of Mn in test solutions are presented in Figs. 2, 3 and 4, respectively. In the figures, the Roman numeral under the abscissa shows the position of each compartment in the transport box, and an asterisked numeral indicates the compartment supplied with a radioisotope-labelled test solution. Experimental treatments are presented under the numerals. A shadowed bar indicates the amount of Mn in the roots and an empty bar shows that in the solution of each compartment. The total amount of Mn absorbed is also presented in the figures.

From the data presented in Figs. 2 to 4, it is evident that the addition of 500 μM Ca to the test solution decreased Mn absorption and translocation in excised roots, as compared with the results in the absence of Ca.

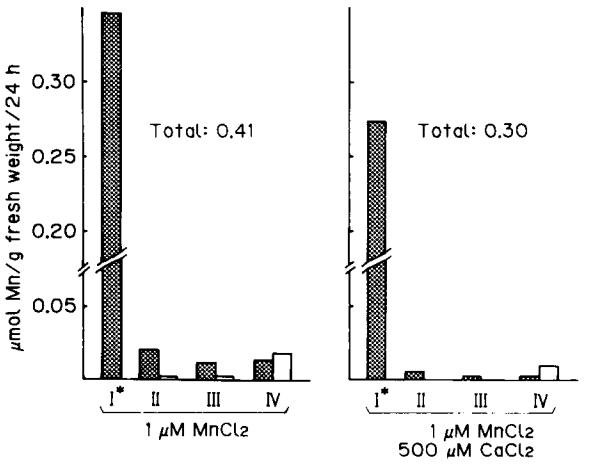

Fig. 2. Effect of Ca on the absorption and translocation of Mn in excised barley roots (Mn 1 μM). An *asterisked numeral* indicates the compartment supplied with a radioisotope-labelled test solution. *Shadowed bars* show the amount of Mn in the roots and *empty bars* that in the solution.

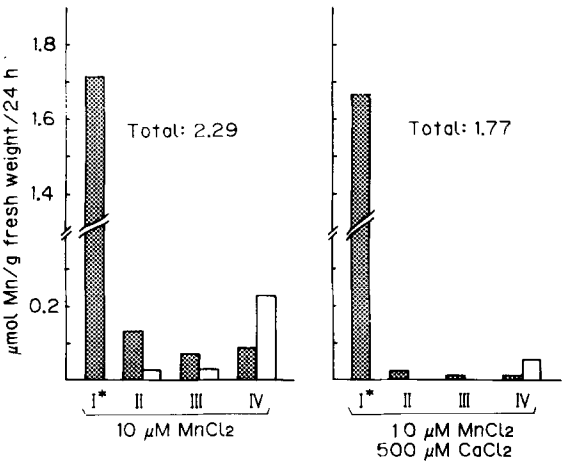

Fig. 3. Effect of Ca on the absorption and translocation of Mn in excised barley roots (Mn 10 μM). The *symbols* are the same as in Fig. 2.

The distribution pattern of ions in an excised plant root can be classified qualitatively as follows: the portion of ions retained in the apex, the portion translocated and redistributed in the root, the portion moved outward across the cortex and the portion exuded into the external solution through the cut end of the root. These four portions are named tentatively "accumulation", "redistribution", "cortical efflux" and "xylem exudation", respectively, and represented schematically in Fig. 5.

With this distribution pattern in mind, the results presented in Figs. 2, 3 and 4 are summarized in Table 1, with an emphasis on comparison between the absence and presence of Ca in test solutions. In Table 1, the

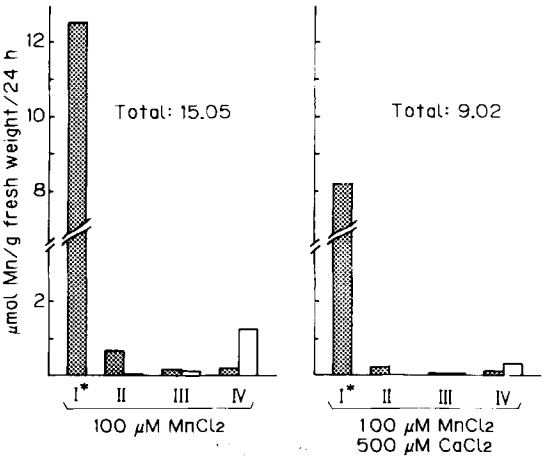

Fig. 4. Effect of Ca on the absorption and translocation of Mn in excised barley roots (Mn 100 μM). The *symbols* are the same as in Fig. 2.

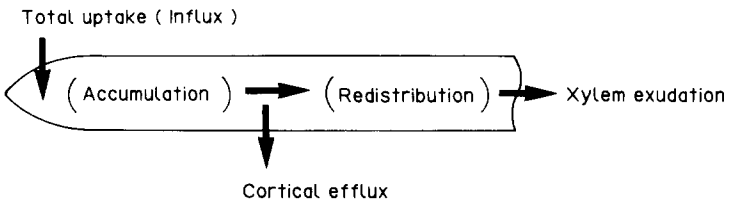

Fig. 5. Schematic representation of the absorption and distribution of ions in excised roots.

results are shown as the percent of each portion to total Mn absorbed. As Table 1 illustrates Ca (500 μM) in test solutions inhibited the absorption and decreased severely the translocation of Mn in excised roots.

The effects of various concentrations of Ca on the absorption and translocation of Mn were examined at 10 μM treatment of Mn in test solutions (Table 2). Results clearly show that Ca at 5 or 50 μM in test solutions had no effect on the absorption of Mn, while 500 μM of Ca inhibited Mn absorption. A 50 μM Ca concentration reduced slightly the translocation of Mn and 500 μM Ca decreased it drastically.

Zn absorption and translocation in excised roots

Using various concentrations of Zn, its absorption and translocation

Table 1. Percent expression of the absorption and distribution of Mn in excised barley roots in the absence and presence of Ca

Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Mn	Ca					
1	0	100 (0.41)*	84	11	0.9	4
	500	100 (0.30)	93	4	0.1	4
10	0	100 (2.29)	75	13	2	10
	500	100 (1.77)	94	2	0.1	3
100	0	100 (15.1)	83	7	1	9
	500	100 (9.0)	91	4	0.7	4

* Numbers in parentheses show total amounts of Mn absorbed as μmol/g fresh weight/24 h.

Table 2. Percent expression of the absorption and distribution of Mn in excised barley roots at various Ca concentrations

Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Mn	Ca					
10	0	100 (2.29)*	75	13	2	10
10	5	100 (2.58)	75	14	2	9
10	50	100 (2.33)	81	8	0.7	11
10	500	100 (1.77)	94	2	0.1	3

* Numbers in parentheses show total amounts of Mn absorbed as μmol/g fresh weight/24 h.

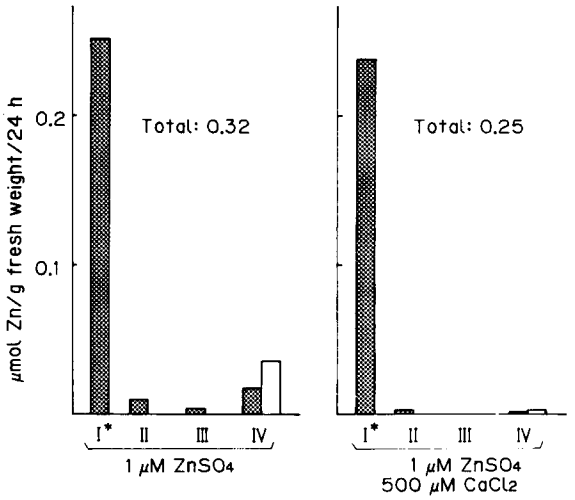

Fig. 6. Effect of Ca on the absorption and translocation of Zn in excised barley roots (Zn 1 μM). An *asterisked numeral* indicates the compartment supplied with a radioisotope-labelled test solution. *Shadowed bars* show the amount of Zn in the roots and *empty bars* that in the solution.

in excised barley roots were examined, as in the case of Mn, in the absence and presence of Ca (500 μM). Figs. 6, 7 and 8 show the results of 1, 10 and 100 μM Zn treatments in test solutions. From Figs. 6 to 8, it is clear that the addition of 500 μM Ca to the test solution decreased Zn absorption and translocation in excised roots.

Figs. 6 to 8 are summarized in Table 3 with an emphasis on comparison between the absence and presence of Ca in test solution, and the

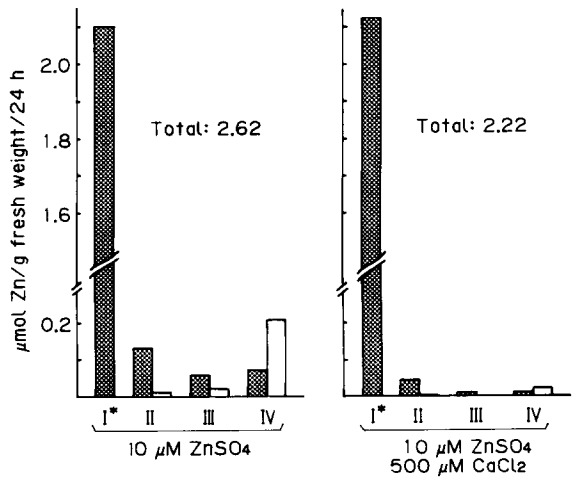

Fig. 7. Effect of Ca on the absorption and translocation of Zn in excised barley roots (Zn 10 μM). The *symbols* are the same as in Fig. 6.

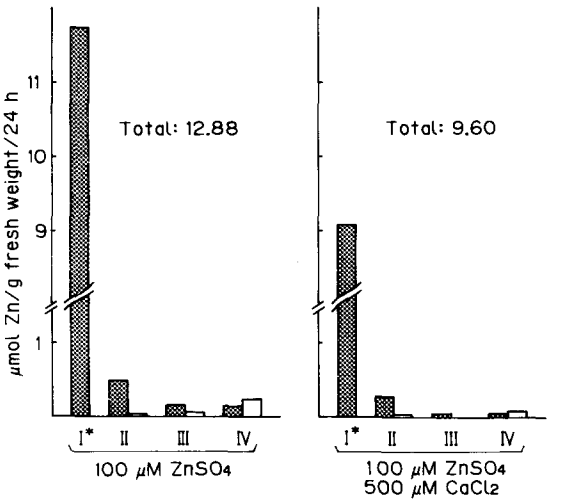

Fig. 8. Effect of Ca on the absorption and translocation of Zn in excised barley roots (Zn 100 μM). The *symbols* are the same as in Fig. 6.

results are shown as the percent of each portion to total Zn absorbed. Table 3 illustrated clearly that a 500 μM concentration of Ca in test solution inhibited Zn absorption and translocation. These functions decreased with increasing concentrations of Ca in test solutions (Table 4).

Cd absorption and translocation in excised roots

Effect of Ca on the absorption and translocation of Cd in excised roots. The absorption and translocation of Cd in excised barley roots were examined using various concentrations of Cd in the absence and presence of Ca. The results for 1, 10 and 100 μM treatments of Cd in test

Table 3. Percent expression of the absorption and distribution of Zn in excised barley roots in the absence and presence of Ca

Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Zn	Ca					
1	0	100 (0.32)*	78	10	0.5	11
	500	100 (0.25)	97	2	0	1
10	0	100 (2.62)	80	10	1	8
	500	100 (2.22)	96	3	0.1	1
100	0	100 (12.9)	91	6	0.7	2
	500	100 (9.6)	95	4	0.4	1

* Numbers in parentheses show total amounts of Zn absorbed as μmol/g fresh weight/24 h.

Table 4. Percent expression of the absorption and distribution of Zn in excised barley roots at various Ca concentrations

Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Zn	Ca					
10	0	100 (2.62)*	80	10	1	8
10	5	100 (2.48)	84	8	0.8	7
10	50	100 (2.29)	92	4	0.4	3
10	500	100 (2.22)	96	3	0.1	1

* Numbers in parentheses show total amounts of Zn absorbed as μmol/g fresh weight/24 h.

solutions are shown in Figs. 9, 10 and 11, respectively. Ca, at various concentrations up to 500 μM, was added to test solutions at a 10 μM Cd concentration (Fig. 10).

The addition of Ca to test solutions drastically decreased the absorption of Cd (Figs. 9–11), and this inhibition became severer with increasing Ca concentrations (Fig. 10). On the other hand, it appears that Ca increased the translocation of Cd in excised roots, except at a 100 μM Cd concentration (Figs. 9–11).

As we mentioned previously, the distribution pattern of ions in excised roots is classified qualitatively into four portions (Fig. 5). Keeping in mind this distribution pattern, the data in Fig. 10 are summarized in Table 5. In Table 5, the results are shown as the percent of each portion of ions to total Cd absorbed.

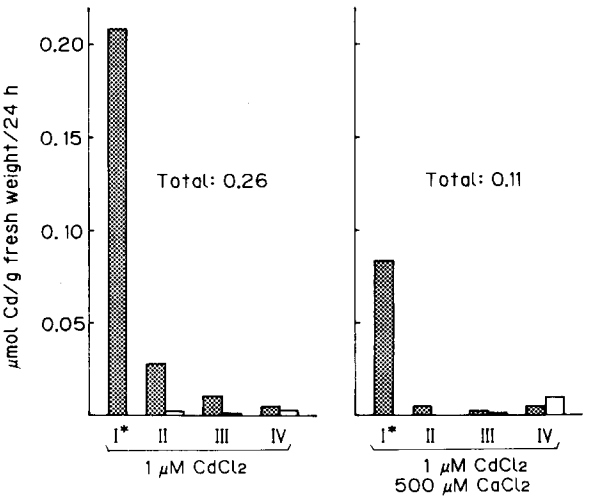

Fig. 9. Effect of Ca on the absorption and translocation of Cd in excised barley roots (Cd 1 μM). An *asterisked numeral* indicates the compartment supplied with a radioisotope-labelled test solution. *Shadowed bars* show the amount of Cd in the roots and *empty bars* that in the solution.

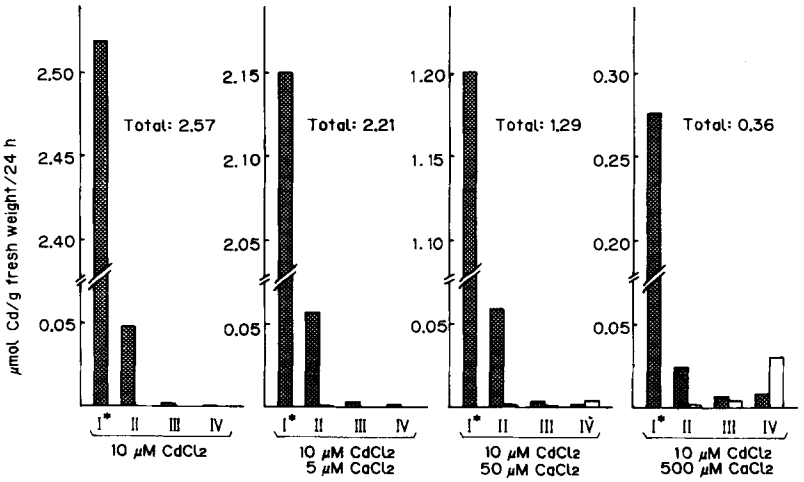

Fig. 10. Effect of Ca on the absorption and translocation of Cd in excised barley roots (Cd 10 μM). The *symbols* are the same as in Fig. 9.

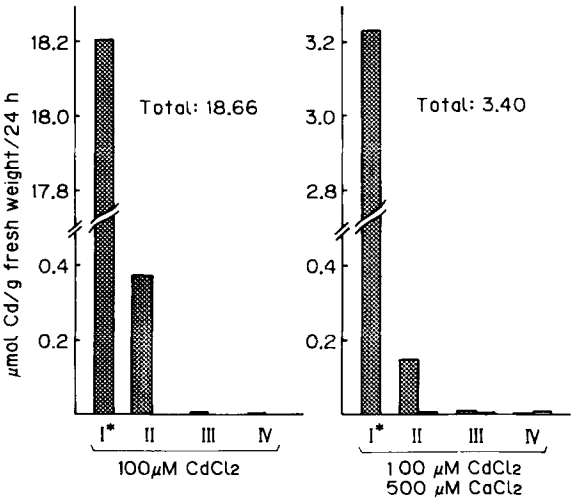

Fig. 11. Effect of Ca on the absorption and translocation of Cd in excised barley roots (Cd 100 μM). The *symbols* are the same as in Fig. 9.

Ca in test solutions inhibited the absorption, while it accelerated the translocation of Cd, especially "xylem exudation" across the cut end of the roots into the external solution (Table 5). These effects of Ca on Cd absorption and translocation became bigger with increasing Ca concentrations in test solutions.

Effects of metabolic inhibitors on the Ca-induced acceleration of Cd translocation. The accelerating effect of Ca on Cd translocation in ex-

Table 5. Percent expression of the absorption and distribution of Cd in excised barley roots at various Ca concentrations

Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Cd	Ca					
10	0	100 (2.57)*	98	2	0	0
10	5	100 (2.21)	97	3	0	0
10	50	100 (1.29)	94	5	0.2	0.3
10	500	100 (0.36)	78	12	2	9

* Numbers in parentheses show total amounts of Cd absorbed as μmol/g fresh weight/24 h.

cised roots was examined in the absence or presence of the metabolic inhibitors, 2,4-dinitrophenol (DNP), chloramphenicol (CP) and p-chloromercuribenzene sulphonic acid (PCMBS). The results are shown in Table 6.

It was found that the accelerating effect of Ca on Cd translocation, especially xylem exudation, decreased with the addition of 10 μM DNP, though 1 μM DNP had no effect. CP and PCMBS had no effect or slightly stimulating effects on Cd translocation (Table 6).

Distribution patterns of Cd in excised roots from barley plants grown under varied culture conditions. In the present experiment, barley plants were usually supplied with 0.25 mM $CaSO_4$ for two days and, subsequently, with nutrient solution for one day during the growing period. To investigate the effect of culture conditions on the distribution patterns of Cd in excised roots, barley plants were grown with 0.25 mM $CaSO_4$ for the entire growing period and no addition of nutrient solution on the last day. Excised roots from plants grown without nutrient solution were compared to those from plants supplied with nutrient solution. The results are presented in Table 7, with an emphasis on comparison between the absence and presence of Ca.

As was seen earlier (Table 5), in excised roots supplied with nutrient solution on the last day of the growth period, the addition of Ca to test solutions inhibited Cd absorption, but accelerated its translocation. By contrast, in excised roots supplied with $CaSO_4$ solution (without nutrient solution), the addition of Ca did not accelerate Cd translocation, though inhibited its absorption.

Discusion

It is well known that Ca plays an essential role in the ion absorption of plants, especially in the selectivity of cation absorption[14,15], and that it inhibits the absorption of heavy metals by plant roots[5,7,10,25]. In addi-

Table 6. Percent expression of the absorption and distribution of Cd in excised barley roots in the absence and presence of Ca and metabolic inhibitors

Treatment (μM)				Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
Cd	Ca	Inhibitor						
10	0	DNP	0	100 (2.57)*	98	2	0	0
10	500	DNP	0	100 (0.34)	74	12	0.8	14
10	500	DNP	1	100 (0.30)	72	13	0.9	14
10	500	DNP	10	100 (0.25)	86	11	0.4	3
10	0	CP	0	100 (2.56)	98	2	0	0
10	500	CP	0	100 (0.34)	78	11	2	10
10	500	CP	5	100 (0.38)	73	10	1	16
10	500	CP	100	100 (0.40)	68	12	1	18
10	0	PCMBS	0	100 (2.88)	98	2	0	0
10	500	PCMBS	0	100 (0.33)	74	12	1	13
10	500	PCMBS	10	100 (0.35)	64	13	2	21
10	500	PCMBS	100	100 (0.31)	71	12	1	16

* Numbers in parentheses show total amounts of Cd absorbed as μmol/g fresh weight/24 h.

Table 7. Percent expression of the absorption and distribution of Cd in excised barley roots grown under varied culture conditions

Culture condition*	Treatment (μM)		Total uptake (%)	Accumulation (%)	Redistribution (%)	Cortical efflux (%)	Xylem exudation (%)
	Cd	Ca					
A	10	0	100 (2.54)**	98	2	0	0
	10	500	100 (0.35)	78	11	1	10
B	10	0	100 (2.00)	97	3	0	0
	10	500	100 (0.56)	96	3	0.2	0.1

* A: $CaSO_4$ → Nutrient solution; B: $CaSO_4$ → $CaSO_4$

** Numbers in parentheses show total amounts of Cd absorbed as μmol/g fresh weight/24 h.

tion, it was reported that Ca depressed the translocation of Zn from roots to shoots[10,25].

In the present investigation, it is clear that Mn absorption in excised barley roots was inhibited (Figs. 2–4, and Tables 1 and 2), as was that of Zn (Figs. 6–8, and Tables 3 and 4), by the presence of Ca in the test solutions. This presence, moreover, inhibited the translocation of Mn and Zn, the effect being slightly severer in the case of Zn.

The amount of ions translocated in excised roots is regarded as a sum of ions participating in the "redistribution", "cortical efflux" and "xylem exudation" in Fig. 5. When we compare the effect of Ca on the total absorption to those on the translocation of Mn or Zn, we see that its inhibitory effects were more drastic on the latter function (Tables 1–4). These results provide a possible explanation for the effect of Ca on the transport pattern of heavy metals in intact plants.

On the other hand, Cd is very similar in its chemical properties to Zn. However, Cd is not an essential element and unlike Zn, is toxic to both plants and animals[20]. There have been many reports on the absorption of Cd by plant roots[8,9,12] and intact plants[9,11,13,22]. During the course of studies on Cd absorption, it has been found that Ca in nutrient solution depressed the Cd absorption in intact plants[13] and excised plant roots[12].

Our investigation revealed that while Ca in test solutions inhibited Cd absorption (Figs. 9–11 and Table 5), it accelerated Cd translocation in excised roots at 1 and 10 μM Cd concentrations (Figs. 9 and 10, and Table 5). The results contrast with those of studies on Mn and Zn mentioned above, in which Ca inhibited both absorption and translocation of these elements.

At a 100 μM Cd concentration, the accelerating effect of Ca on the Cd translocation was negligibly small (Fig. 11). This may be due to the toxicity of Cd itself at high concentrations.

There are some conflicting results on Cd absorption in plants, in which one report concluded that the Cd absorption process was non-metabolic[8], while another suggested active absorption at low external concentra-

tions of Cd^9. In the present experiment, the addition of DNP reduced the accelerating effect of Ca on Cd translocation in excised roots, though CP and PCMBS had no effect (Table 6). These results suggest that this accelerating effect might be involved in the metabolic pathway, perhaps in energy production. Further investigations should be carried out.

In excised roots grown under culture conditions without a nutrient solution (with only $CaSO_4$ solution) Ca did not accelerate Cd translocation (Table 7). In the future, a detailed examination of the effects of culture conditions, especially nutrient supply, on Cd translocation would be of value and interest.

Acknowledgements The multi-compartment transport box used in the present investigation was designed fundamentally by J M Stassart, Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel. The authors thank Stassart for his valuable suggestions. The authors also wish to thank Miss E Kimoto for her excellent technical assistance.

The present work was partly supported by a Grant-in-Aid for Scientific Research (No. 60560073) from the Ministry of Education, Science and Culture, Japan.

References

1 Bajaj Y P S, Rathore V S, Wittwer S H and Adams M W 1970 Effect of dimethyl sulfoxide on $zinc^{65}$ uptake, respiration, and RNA and protein metabolism in bean (*Phaseolus vulgaris*) tissues. Am. J. Bot. 57, 794–799.

2 Bowen J E 1973 Kinetics of zinc absorption by excised roots of two sugarcane clones. Plant and Soil 39, 125–129.

3 Braunsberg H and Guyver A 1965 Automatic liquid scintillation counting of high-energy β emitters in tissue slices and aqueous solutions in the absence of organic scintillator. Anal. Biochem. 10, 86–95.

4 Chandel A S and Saxena M C 1980 Mechanism of uptake and translocation of zinc by pea plants (*Pisum sativum* L.). Plant and Soil 56, 343–353.

5 Chaudhry F M and Loneragan J F 1972 Zinc absorption by wheat seedlings: I. Inhibition by macronutrient ions in short-term experiments and its relevance to long-term zinc nutrition. Soil Sci. Soc. Amer. Proc. 36, 323–327.

6 Chaudhry F M and Loneragan J F 1972 Zinc absorption by wheat seedlings: II. Inhibition by hydrogen ions and by micronutrient cations. Soil Sci. Soc. Am. Proc. 36, 327–331.

7 Chaudhry F M and Loneragan J F 1972 Zinc absorption by wheat seedlings and the nature of its inhibition by alkaline earth cations. J. Exp. Bot. 23, 552–560.

8 Cutler J M and Rains D W 1974 Characterization of cadmium uptake by plant tissue. Plant Physiol. 54, 67–71.

9 Fujimoto T and Uchida Y 1979 Cadmium absorption by rice plants. I. Mode of the absorption. Soil Sci. Plant Nutr. 25, 407–415.

10 Giordano P M, Noggle J C and Mortvedt J J 1974 Zinc uptake by rice, as affected by metabolic inhibitors and competing cations. Plant and Soil 41, 637–646.

11 Greger M and Lindberg S 1986 Effects of Cd^{2+} and EDTA on young sugar beets (*Beta vulgaris*). I. Cd^{2+} uptake and sugar accumulation. Physiol Plant. 66, 69–74.

12 Hardiman R T and Jacoby B 1984 Absorption and translocation of Cd in bush beans (*Phaseolus vulgaris*). Physiol. Plant. 61, 670–674.

13 Iwai I, Hara T and Sonoda Y 1975 Factors affecting cadmium uptake by the corn plant. Soil Sci. Plant Nutr. 21, 37–46.

14 Jacobson L, Hannapel R J, Moore D P and Schaedle M 1961 Influence of calcium on selectivity of ion absorption process. Plant Physiol. 36, 58–61.

15 Kawasaki T and Hori S 1973 The role of calcium in selective cation uptake by plant roots. II. Effects of temperature, desorption treatment and sodium salt on rubidium uptake. Ber. Ohara Inst. Landw. Biol. Okayama Univ. 16, 19–28.
16 Kawasaki T, Shimizu G and Moritsugu M 1983 Effects of high concentrations of sodium chloride and polyethylene glycol on the growth and ion absorption in plants. II. Multi-compartment transport box experiment with excised roots of barley. Plant and Soil 75, 87–93.
17 Kawasaki T, Moritsugu M and Shimizu G 1984 The absorption and translocation of ions in excised barley roots: A multi-compartment transport box experiment. Soil Sci. Plant Nutr. 30, 417–425.
18 Kobayashi J 1969 An investigation on the cause of "itai-itai disease". I–III. Kagaku (Science) 39, 286–293, 369–375, 424–429 (*In Japanese*)
19 Landi S and Fagioli F 1983 Efficiency of manganese and copper uptake by excised roots of maize genotypes. J. Plant Nutr. 6, 957–970.
20 Mengel K and Kirkby E A 1978 Principles of Plant Nutrition, Intern. Potash Inst., Berne, p. 441–458 and 519–520.
21 Page E R and Dainty J 1964 Manganese uptake by excised oat roots. J. Exp. Bot. 15, 428–443.
22 Petit C M, Ringoet A and Myttenaere C 1978 Stimulation of cadmium uptake in relation to the cadmium content of plants. Plant Physiol. 62, 554–557.
23 Ramani S and Kannan S 1975 Manganese absorption and transport in rice. Physiol. Plant. 33, 133–137.
24 Riedell W E and Schmid W E 1986 Physiological and cytological aspects of manganese toxicity in barley seedlings. J. Plant Nutr. 9, 57–66.
25 Sadana U S and Takkar P N 1983 Effect of calcium and magnesium on ^{65}Zn absorption and translocation in rice seedlings. J. Plant Nutr. 6, 705–715.
26 Schmid W, Haag H P and Epstein E 1965 Absorption of zinc by excised barley roots. Physiol. Plant. 18, 860–869.
27 Veltrup W 1978 Characteristics of zinc uptake by barley roots. Physiol. Plant. 42, 190–194.