Distribution and Translocation of $^{141}$Ce (III) in Horseradish

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• Background and Aims Rare earth elements (REEs) are used in agriculture and a large amount of them contaminate the environment and enter foods. The distribution and translocation of $^{141}$Ce (III) in horseradish was investigated in order to help understand the biochemical behaviour and toxic mechanism of REEs in plants.
• Methods The distribution and translocation of $^{141}$Ce (III) in horseradish were investigated using autoradiography, liquid scintillation counting (LSC) and electron microscopic autoradiography (EMARG) techniques. The contents of $^{141}$Ce (III) and nutrient elements were analysed using an inductively coupled plasma-atomic emission spectrometer (ICP-AES).
• Results The results from autoradiography and LSC indicated that $^{141}$Ce (III) could be absorbed by horseradish and transferred from the leaf to the leaf-stalk and then to the root. The content of $^{141}$Ce (III) in different parts of horseradish was as follows: root > leaf-stalk > leaf. The uptake rates of $^{141}$Ce (III) in horseradish changed with the different organs and time. The content of $^{141}$Ce (III) in developing leaves was greater than that in mature leaves. The results from EMARG indicated that $^{141}$Ce (III) could penetrate through the cell membrane and enter the mesophyll cells, being present in both extra- and intra-cellular deposits. The contents of macronutrients in horseradish were decreased by $^{141}$Ce (III) treatment.
• Conclusions $^{141}$Ce (III) can be absorbed and transferred between organs of horseradish with time, and the distribution was found to be different at different growth stages. $^{141}$Ce (III) can enter the mesophyll cells via apoplast and symplast channels or via plasmodesmata. $^{141}$Ce (III) can disturb the metabolism of macronutrients in horseradish.

Key words: Horseradish, Armoracia rusticana, cerium, $^{141}$Ce (III), translocation, distribution, radioisotope tracer technique.

INTRODUCTION

From the 1970s onwards, it was found that the yield and quality of crops such as wheat, rice, maize, mungbean, and many others could be significantly improved by using rare earth microfertilizers (Buckingham et al., 1999; Diatloff et al., 1999; Hong et al., 2000). It was well known that a large amount of these rare earth elements (REEs) entered the environment and foods, leading to environmental contamination and the accumulation of the REEs in the food chain (Volokh et al., 1990). Thus, many papers have reported the environmental and ecological effects of REEs (Markert and Li, 1991; Tyler, 2004; Huang et al., 2005). However, the biochemical behaviour of REEs in the soil–plant system is still not fully clear.

The distribution of the REEs in plant cells has been investigated in order to understand the biochemical behaviour of the REEs in the soil–plant system (Xu et al., 2002; Showler et al., 2006; Tagami and Uchida, 2006; Shtangeeva and Ayrault, 2007). However, there is very little information about whether rare earth ions can enter into plant cells and, if so, how they are distributed within them. In addition, as the concentrations of REEs are generally very low in different plant organs, conventional analytical methods have not been able to provide the desired sensitivity and accuracy. It has been reported that the radioisotopic tracing technique can be used to analyse the distribution and translocation of metal ions in the whole plant (Lin et al., 1995; Tausz et al., 2003; Bystrzejewska-Piotrowska and Urban, 2004; Mosquera et al., 2006) and the passage of ions across membranes in intact cells, synaptosomes and related systems (Lukas and Cullen, 1988; Villarroya et al., 1998; Fitch and Daly, 2005). Furthermore, the autoradiographic technique could be applied in order to obtain imaging of the uptake, translocation and distribution of radioisotopes (Mäkelä et al., 1996; Soudek et al., 2006). In addition, liquid scintillation counting (LSC) is an effective technique for the determination of radionuclides, because of high counting efficiency, easy sample preparation and easy automatization (Gómez-Scarbor et al., 1996). Thus, using radioisotopes of REEs as tracers is potentially a good way to study the behaviour of REEs in plants.

Horseradish (Armoracia rusticana) is a perennial herb of the cruciferae family, native to central and south Europe and naturalized in many parts of North America. In this paper, the translocation and distribution of $^{141}$Ce (III) in horseradish were investigated, together with the effect of $^{141}$Ce (III) on the uptake of macronutrients...
such as K, Ca and Mg, by using autoradiography, LSC and electron microscopic autoradiography (EMARG) techniques. The aim of the study was to provide valuable information towards understanding the effects of REEs on plant growth, environmental toxicology and food safety.

MATERIALS AND METHODS

Preparation of radioactive cerium solution

$^{141}\text{CeO}_2$, as pale yellow powder, was supplied by Beijing Atom High Tech Co., Ltd in China. Its specific radioactivity concentration was $3.7 \times 10^5 \text{ Bq mg}^{-1}$ and its radiochemical purity was greater than 95%. Prior to use, it was chemically transformed with $\text{H}_2\text{O}_2$ and $\text{HNO}_3$ into $^{141}\text{Ce(NO}_3)_3$ form as follows:

$$2^{141}\text{CeO}_2 + \text{H}_2\text{O}_2 = ^{141}\text{Ce}_2\text{O}_3 + \text{H}_2\text{O} + \text{O}_2$$

$$^{141}\text{Ce}_2\text{O}_3 + 6 \text{HNO}_3 = 2^{141}\text{Ce(NO}_3)_3 + 3\text{H}_2\text{O}$$

Then, 1.1 mL of the $^{141}\text{Ce(NO}_3)_3$ solution was diluted to 15 mL with $\text{H}_2\text{O}$. The diluted solution with $2.4 \times 10^8 \text{ c.p.m. mL}^{-1}$ specific radioactivity was used for the experiments.

Plant culture and treatments

Horseradish, Armoracia rusticana, was grown in a glasshouse at 20–25°C and a 16-h photoperiod with 300 $\mu\text{mol m}^{-2}\text{s}^{-1}$ irradiance. The horseradish was planted in plastic pots in the middle of March. The diameter of the plastic pot was 40 cm and three plants grew in each pot.

When the horseradish had reached a mature stage, 1 mL of the diluted solution of $^{141}\text{Ce (III)}$ was daubed on a leaf surface of each treated plant (25 October). This leaf is referred to as the labelled leaf, and the other leaves and organs without the treatment of $^{141}\text{Ce (III)}$ are referred to as the unlabelled organs. Three pots of horseradish samples were harvested on 1, 2, 8 and 16 days after the treatment with $^{141}\text{Ce (III)}$. Samples of the leaves, leaf-stalks and roots from one pot were collected for autoradiography analysis, and the other two pots were used for radioactive determination, in which three samples were collected from the different plants in the pots.

Autoradiography analysis of translocation of $^{141}\text{Ce(III)}$

The samples of leaves, leaf-stalks and roots of $^{141}\text{Ce (III)}$-teated horseradish were cut, washed with non-radioactive Ce(NO$_3$)$_3$ solution in order to prevent $^{141}\text{Ce (III)}$ from diffusing out in the organs, and then washed with EDTA solution. Then, the samples were pressed between two filter papers and dried at 70°C for 8 h. The samples were transferred to a Valumax cassette ($24 \times 30$ cm) and put on X-ray monitoring film. The film was exposed for different durations of time depending on the type of nuclide, and then developed.

Determination of radioactivity of $^{141}\text{Ce(III)}$ in different organs

The samples of the leaves, leaf-stalks and roots were cut and then washed with non-radioactive Ce(NO$_3$)$_3$ solution and EDTA solution as above. The samples were dried overnight at 70–80°C and ground to a fine powder. A 20-mg sample of the powder was placed into a LSC vial, digested in 100 $\mu\text{L}$ HNO$_3/\text{HClO}_4$ (5 : 1 v/v) and 200 $\mu\text{L}$ H$_2\text{O}_2$ and incubated at 70–80°C for 30–45 min. Then 5 mL of hydrophilic scintillating solution [5 g of PPO (2,5-diphenyloxazole), 665 mL xylene and 335 mL ethylenediethanol ether] were added into the vial and mixed thoroughly using a vortex for 30 min. Finally, the radioactivity of the sample was determined using a liquid scintillation counter (Beckman LS-6500). The radioactivity of each sample was calculated using the calibration of
sample quenching, radioactive decay and background. The background value was the radioactivity of the hydrophilic scintillating solution.

Each sample was repeated three times, and average values are given here. The mean deviation of the radioactivity measurements was about 0.5%.

EMARG of $^{141}$Ce(III)

Young horseradish leaves were treated with $^{141}$Ce (III) for 8 d. The leaf was then cut as a regular section of 1.5 × 2 mm size, and as an ultra-thin section of approx. 60 nm for the electron microscope observation (Reichert Ultracut E ultramicrotome). Nuclear emulsion (Technical Institute of Physics and Chemistry, Chinese Academy of Science) was painted on the leaf section and, following development and fixation, the distribution of rare earth ions could be observed using an electron microscope (Feig and Harting, 1992). The leaf sections for TEM measurements were prepared according to the method of Santos et al. (2004), and images were observed with a H-600 TEM (Hitachi Company, Japan).

Measurement of content of macronutrients (K, Ca, Mg) in roots

The roots of horseradish that had been treated with $^{141}$Ce (III) were cut and washed with triple-distilled water. The cut roots were dried overnight at 70–80 °C and ground to a fine powder. A 0.5-g sample of the powder was digested in a Microwave Digestion System CEM 2000 in a closed Teflon bomb according Chojnacka et al. (2004). The reagent composition and digestion conditions were chosen in order to achieve complete mineralization and decomposition of the solid phase into the liquid phase. After digestion, the solution was adjusted to 50 mL with triple-distilled water. The macronutrient contents (K, Ca, Mg) of digested samples were determined by ICP-AES.

The experimental data were analysed using a LSD test (at $P < 0.05$).
RESULTS AND DISCUSSION

Figure 1 shows normal photographs and autoradiographic images of leaf-stalks connected to labelled leaves, together with the corresponding radioactivities on the 2nd and 8th days. It can be seen that almost no autoradiographic images were observed in the unlabelled organs 2 d after the treatment, indicating that very little $^{141}$Ce(III) moved from the labelled leaf to other organs in this time. However, 8 d after the treatment with $^{141}$Ce(III), the radioactivities of the unlabelled organs were clearly higher than the background (Fig. 2C), indicating that large amounts of $^{141}$Ce(III) had been moved to the labelled leaf to the other organs in this time. On the 16th day, every unlabelled organ produced autoradiographic images (Fig. 3A) and high radioactivity (Fig. 3B), suggesting that the distribution of $^{141}$Ce(III) in unlabelled organs changed with the time, thus illustrating the transfer of $^{141}$Ce(III) within horseradish.

It can be seen from Fig. 2C and Fig. 3B that the radioactivity in the root was the highest among all the organs, while the radioactivity in leaf-stalk was higher than that in the leaf. Thus, it was evident that $^{141}$Ce(III) had uneven distribution within horseradish and its content showed the following order: root > leaf-stalk > leaf.

In order to further examine this phenomenon, the radioactivities and uptake rates of $^{141}$Ce(III) in the different organs at different times were determined (Table 1). It was found that the radioactivities of $^{141}$Ce(III) in the different organs differed at any given time. For example, the radioactivity in the labelled leaf on the 2nd day was approx. $1.10 \times 10^8$ cpm, whereas it was approx. $1.70 \times 10^6$ cpm in the leaf-stalk connected to that leaf. The radioactivities of $^{141}$Ce(III) in unlabelled roots, leaf-stalks and leaves were $2.60 \times 10^4$, $1.80 \times 10^4$ and $9.00 \times 10^3$ cpm, respectively. The radioactivities in the unlabelled organs on the 16th day were higher than those on the 2nd day, and had the following values (in descending order): $5.10 \times 10^6$ cpm for the unlabelled roots; $2.80 \times 10^6$ cpm for the unlabelled leaf-stalks; $1.70 \times 10^6$ cpm for the unlabelled leaves; and $1.30 \times 10^6$ cpm for the leaf-stalks connected with the labelled leaves. These results indicate that $^{141}$Ce(III) has clearly been absorbed by horseradish and then moved from the labelled leaf to the unlabelled organs.

The radioactivities of $^{141}$Ce(III) in the labelled leaves and the leaf-stalks connected with them reached maximum values between the 2nd and 8th days, and then they decreased to low levels due to the translocation of $^{141}$Ce(III). The $^{141}$Ce(III) uptake rates of the unlabelled organs increased with time. On the 2nd day, the maximum uptake rate of the labelled leaf was 45.8 % and then it decreased significantly with time (Table 1). Meanwhile, the uptake rates of the unlabelled organs increased to some extent. Therefore, it can be concluded from the results that the distribution of $^{141}$Ce(III) in horseradish varied between different organs and with time.

The radioactivities of developing and mature unlabelled leaves and leaf-stalks of horseradish on the 8th and 16th day are shown in Fig. 4. It can be seen that the radioactivities in developing leaves and leaf-stalks are higher
leaves of horseradish on (A) the 8th and (B) the 16th day after treatment. Metabolism of the developing parts of the plant is fast, $^{141}$Ce(III) is related to the metabolism of the plant. The reason is not clear, but it may be because the translocation of $^{141}$Ce(III) is related to the metabolism of the plant. The specific activity of each organ tested/initial activity of $^{141}$Ce(III).

Table 1. Absorption of $^{141}$Ce in horseradish

<table>
<thead>
<tr>
<th>Time (d)</th>
<th>Labelled leaf</th>
<th>Leaf-stalk connected to labelled leaf</th>
<th>Root</th>
<th>Leaf-stalk</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity (cpm g$^{-1}$)</td>
<td>Specific activity (cpm g$^{-1}$)</td>
<td>Specific activity (cpm g$^{-1}$)</td>
<td>Specific activity (cpm g$^{-1}$)</td>
<td>Specific activity (cpm g$^{-1}$)</td>
</tr>
<tr>
<td>Control</td>
<td>120 ± 2$^a$</td>
<td>118 ± 1$^a$</td>
<td>101 ± 3$^a$</td>
<td>121 ± 1$^a$</td>
<td>116 ± 2$^a$</td>
</tr>
<tr>
<td>1</td>
<td>0$^a$</td>
<td>0$^a$</td>
<td>1-70 ± 0.03 $\times 10^3$</td>
<td>9-00 ± 0.03 $\times 10^3$</td>
<td>9-00 ± 0.03 $\times 10^3$</td>
</tr>
<tr>
<td>2</td>
<td>36-3 ± 0.1$^a$</td>
<td>0-333 ± 0.002$^b$</td>
<td>7-00 ± 0.05 $\times 10^3$</td>
<td>4-00 ± 0.04 $\times 10^3$</td>
<td>2-00 ± 0.05 $\times 10^3$</td>
</tr>
<tr>
<td>8</td>
<td>45-8 ± 0.08$^a$</td>
<td>0-708 ± 0.005$^d$</td>
<td>1-10 ± 0.09 $\times 10^{-2}$</td>
<td>8-00 ± 0.03 $\times 10^{-3}$</td>
<td>4-00 ± 0.09 $\times 10^{-3}$</td>
</tr>
<tr>
<td>16</td>
<td>31-3 ± 0.2$^c$</td>
<td>2-96 ± 0.06$^d$</td>
<td>0-417 ± 0.012$^d$</td>
<td>0-242 ± 0.011$^d$</td>
<td>0-108 ± 0.015$^d$</td>
</tr>
</tbody>
</table>

Initial activity of $^{141}$Ce(III) applied to each plant was 2.4 $\times 10^3$ cpm. Uptake rate (%): specific activity of each organ tested/initial activity of $^{141}$Ce(III).

Figure 5 shows electron microscopic autoradiographs of $^{141}$Ce(III) in horseradish cells. It can be seen that $^{141}$Ce(III) (as indicated by black/silver grains) is mainly distributed outside the cell and on the cell wall, and only small amounts of $^{141}$Ce(III) exist on both sides of plasmodesma.

Normal levels for these elements in plants were defined 3500–6600 mg kg$^{-1}$ for K (Zottl and Hüttl, 1989), 2300–5000 mg kg$^{-1}$ for Ca and 500–1300 mg kg$^{-1}$ for Mg (Smidt, 1988).

Figure 5B shows that $^{141}$Ce(III) is largely distributed in the cytoplasm and plasmodesma, and Fig. 5C and D also show a lot of $^{141}$Ce(III) located in cytoplasm. The results demonstrate that $^{141}$Ce(III) can enter into horseradish cells via plasmodesmata, and then it is mainly distributed in intercellular spaces, cell walls, plasmodesmata and cytoplasm, thus indicating that $^{141}$Ce(III) can be absorbed by leaves and then moved via apoplast vessels to the symplast. Similar behaviours of other ions have been also observed (Clarkson, 1993; Overall and Blackman, 1996; Ma and Carol, 2001).

In order to determine if the $^{141}$Ce(III) absorbed by horseradish would affect the distribution of other essential plant nutrients, the contents of K, Ca and Mg in the roots of control and treated plants were measured after 16 d (Fig. 6). Normal levels for these elements in plants were defined 3500–6600 mg kg$^{-1}$ for K (Zottl and Hüttl, 1989), 2300–5000 mg kg$^{-1}$ for Ca and 500–1300 mg kg$^{-1}$ for Mg (Smidt, 1988).

It can be seen from Fig. 6 that in control plants the mean contents of K, Ca and Mg in the roots were 4500, 3000 and 700 mg kg$^{-1}$, respectively, which are similar to the normal levels of these elements in plants. However, the mean contents of K, Ca and Mg in the root of horseradish treated with $^{141}$Ce(III) were only 3000, 2000 and 250 mg kg$^{-1}$, respectively, which is much lower than the normal values. The results indicate that when the content of $^{141}$Ce(III) is higher than the normal level of the plant, the metabolism of these macronutrients in

![Fig. 4. The radioactivities recorded in developing and mature unlabelled leaves of horseradish on (A) the 8th and (B) the 16th day after treatment.](https://academic.oup.com/aob/article-abstract/100/7/1459/216156)
horseradish would be disturbed, potentially leading to nutrient deficiency, inhibition of growth and reduced longevity (Lamppu and Huttunen, 2003).

CONCLUSIONS

The distribution and translocation of a rare earth element, i.e. $^{141}\text{Ce(III)}$, in horseradish were qualitatively and quantitatively investigated using radioisotope tracer techniques. The experimental results lead to the following conclusions.

After $^{141}\text{Ce(III)}$ was applied to the surface of the leaf, it could be absorbed and transferred to the other organs of the plant. The uptake rates of $^{141}\text{Ce(III)}$ varied with the different organs. The content of $^{141}\text{Ce(III)}$ in horseradish organs followed the order of: roots $>$ leaf-stalks $>$ leaves.

The distribution of $^{141}\text{Ce(III)}$ was different at different growth stages. The content of $^{141}\text{Ce(III)}$ in the developing leaves was more than that in mature leaves, illustrating that the translocation of $^{141}\text{Ce(III)}$ in horseradish is related to the metabolism of the plant.

$^{141}\text{Ce(III)}$ can penetrate through cell membranes and enter into the mesophyll cells via apoplastic and symplastic channels in the leaf or via plasmodesmata, and both extracellular and intracellular deposition occurs.

The contents of K, Ca and Mg were decreased in horseradish treated with $^{141}\text{Ce(III)}$, indicating that $^{141}\text{Ce(III)}$ can disturb the metabolism of these macronutrients in the plant.

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LITERATURE CITED


