

**Root-Uptake of C-14 Acetic Acid by Various Plants and C-14 Dynamics
Surrounding the Experimental Tessera - 8127**

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ABSTRACT

Carbon-14 (C-14, $t_{1/2}=5.73\times 10^3$ yrs) from radioactive waste is one of the most important radioactive nuclides for environmental assessment in the context of geological disposal, and understanding the transfer of radioactive elements to plants is essential for public health safety. In order to obtain fundamental knowledge, culture experiments using marigold (*Tagetes patula* L.), tall fescue (*Festuca arundinacea* S.), paddy rice (*Oryza sativa* L.), radish (*Raphanus sativus* L.), and carrot (*Daucus carota* L.) plants were conducted to examine root-uptake and dynamics of C-14 in the laboratory. The C-14 radioactivity in each plant part (e.g. shoot, root, edible part, etc.), medium (e.g. culture solution, sand, etc.), and air was determined. The distribution of C-14 in the plants was visualized using autoradiography. For a comparison, autoradiography was also done using Na-22. Results of the present study indicated that C-14 labeled CO₂ gas was released from the culture solution to the atmosphere. Clear autoradiography images were observed in plants for the shoots and lower roots which were soaked in the culture solution. The upper roots which were not soaked in the culture solution were not clearly imaged. In the radiotracer experiment using Na-22, a clear image was observed for the whole carrot seedling, even including the upper root, on the autoradiography. However, the amounts of C-14 acetic acid absorbed by all the plants through their roots were considered to be very small. Inorganic carbon transformed from C-14 acetic acid would be taken up by plants through the roots, and some fraction of C-14 would be assimilated into the shoots by photosynthesis.

INTRODUCTION

Geological disposal of radioactive waste from nuclear facilities is planned to avoid radiation exposure to the public people. For maintaining public health safety and assessing radiation exposure, it is necessary to clarify pathways on how these radioactive elements are transferred through the biosphere to reach humans via consumption of edible plants. Past studies have reported that soybean and rice plants grown in a nutrient solution containing various radioactive nuclides, such as metal elements, accumulated them in the plant parts [1-3]. Also, radiotracer experiments on vegetable plants [4] and root crops [5] have reported. However, little is known about carbon transfer to plants based on geological disposal of radioactive waste.

Carbon-14 (C-14, $t_{1/2}=5.73\times 10^3$ yrs) from radioactive waste is one of the most important radioactive nuclides for environmental assessment in the context of geological disposal. There are both organic and inorganic carbon forms in radioactive waste, and it has been reported that most organic carbon in radioactive waste is present as carboxylic acids such as acetic acid [6]. It has been generally thought that a major carbon source for plants comes from air as carbon dioxide (CO₂) and then it is assimilated by photosynthesis. However, it is also necessary for assessment of geological disposal to clarify transfer of carbon from underground and absorption of carbon by plant roots. Thus, the present study was conducted to examine plant uptake and assimilation of carbon through plant roots by using C-14 nuclide in the form of acetic acid. The root-uptake of organic carbon as acetic acid by various plants and dynamics of carbon surrounding the experimental tessera (spatially heterogeneous environment) were examined using C-14 nuclide.

MATERIALS AND METHODS

Culture experiments (i.e. hydroponics, sand culture, and chamber experiments) using marigold (*Tagetes patula* L.), tall fescue (*Festuca arundinacea* S.), paddy rice (*Oryza sativa* L.), radish (*Raphanus sativus* L.), and carrot (*Daucus carota* L.) plants were conducted to examine root-uptake of carbon in the form of C-14 acetic acid and dynamics of C-14 surrounding the experimental tessera.

Hydroponics: Marigold, Tall Fescue, Paddy Rice, and Carrot

The set-up for the pot experiment using hydroponics is shown in Fig. I. It had an upper pot and a lower pot. A plug pot was used as the upper pot, and it contained sterilized wet sand (121°C, 1 h, 40 g D.W.). The lower pot was a polyethylene beaker filled with water (180 mL) and covered with aluminum foil to reflect sunlight. One plant was raised in the upper pot. Liquid fertilizer (1 mL, containing NH₄NO₃-N at 80 mg L⁻¹, Ca(NO₃)₂-N at 70 mg L⁻¹, NaH₂PO₄-P at 10 mg L⁻¹, K₂SO₄-K at 100 mg L⁻¹, Ca(NO₃)-Ca at 100 mg L⁻¹, and MgSO₄-Mg at 30 mg L⁻¹) was added to the upper pot every 3 days until the roots had grown long enough to pass through the center opening in the bottom of the upper pot. Then, liquid fertilizer (5 mL) was added to the lower pot every 2 days. C-14 labeled acetic acid, in the form of sodium acetate (1, 2-C-14), was added to the lower pot to give a radioactivity of 19 kBq. Thus, the upper pot covered the upper half of the root (upper root), and the lower half of the root (lower root) was the part that had passed through the opening and was soaked in the culture solution.



Fig. I. Hydroponics experiment set-up.

After addition of the C-14 labeled acetic acid, marigold, tall fescue, and paddy rice were cultivated for 24 h (natural light, 45 k Lux for 16 h; temperature, 29°C; relative humidity, 55 %) in a sunny and well ventilated place which faced a glass wall in a restricted laboratory for radioisotope experiments. Carrot seedling was cultivated in a phytotron for 24 h (artificial light, 10 k Lux for 16 h; 23°C, relative humidity; 40 %). For all the types of plants, the solution level in the lower pot was adjusted every 2 days to keep it 2 cm below the bottom of upper pot in order for each plant to take up air via the roots. Plant cultured with non-labeled solution was arranged next to the C-14 added pots as control, and a non-planted pot also served. To examine the changes of C-14 radioactivity in the culture solution, the solution was collected and mixed with counting solution (Hionic-Fluor), then the radioactivity was measured at different time intervals (2, 4, 8, 24 h) using a liquid scintillation counter (Aloka, LSC-5100). The plants were separated into shoot, upper roots, and lower roots after carefully removing them from the pots. The plant samples were exposed to an imaging plate (K-screen) for 48 h for autoradiography after they had been wrapped in a polyvinylidene chloride film. The distribution of C-14 in the plants was visualized using an imaging analyzer (Bio-Rad Lab., Molecular Imager FX system). For a comparison, autoradiography was also done using carrot seedling and Na-22.

Sand Culture Experiments: Paddy Rice and Radish

The sand culture experiment, a submerged sand culture experiment for rice plant and a general sand culture experiment for radish, were done to determine carbon absorption by the plant and assimilation to the edible parts through roots.

Rice plant seedling was transplanted to the experimental pot (a polyethylene beaker) that was filled with sterilized sand (200 g) and culture solution (160 mL). The depth of ponded solution was maintained at 3 cm for the entire experimental period to simulate the submerged condition of rice paddies. Five mL of liquid fertilizer were added every 3 days until 35 days after

transplanting and every 2 days from 35 days onwards. At the grain-forming stage (62 days after transplanting), C-14 labeled acetic acid was added to the pot mixed with liquid fertilizer (radioactivity: 74 kBq), and plants were grown for 2 months in a sunny and well ventilated place. Plant cultured with non-labeled solution was also arranged next to the C-14 added pots as control.

Radish seedling was transplanted to the experimental pot that was filled with sterilized sand (400 g and 70% water saturation point). Ten mL of liquid fertilizer were added every 2 days for 23 days. At 23 days after transplanting C-14 labeled acetic acid was added to the pot mixed with liquid fertilizer (radioactivity: 74 kBq), and radish was grown for 24 h in the phytotron. Plant cultured with non-labeled solution was also arranged next to the C-14 added pots as control. After the plants were carefully removed from the pots, the distribution of C-14 in the plant parts (shoot, root, and edible parts) was visualized on the autoradiography. Also, edible parts were burned and made inorganic solutions using a sample oxidizer (Packard, Model 307), and then these were measured using a liquid scintillation counter.

Chamber Experiment: Paddy Rice

To determine loss of C-14 as vaporized CO₂, a chamber experiment was performed. Rice seedling was grown until 117 days after transplanting. A test pot (radioactivity: 74 kBq) identical to that of the submerged sand culture experiment was prepared. The test pot was placed in a partially closed chamber made of clear glass. There was a small opening in the center of the chamber top. The chamber covered the whole of the experimental pot and lower 15 cm of the shoots. The upper part of the shoots was able to pass out of the chamber through the top opening. The hole on the top was tightly sealed with laboratory film to avoid gas leakage. A vial filled with sodium hydroxide solution (0.5 M NaOH, 10 mL) was placed in the chamber with the experimental pot to trap CO₂. The rice plant was cultured for 20 days. The radioactivity of C-14 in the NaOH solution in the vial was determined at 0, 2, 4, 8, 12, 16, and 20 days using a liquid scintillation counter. Every time at determination, NaOH solution in the vial was replaced by a fresh amount of solution. The distribution of C-14 in the plant was visualized by autoradiography.

RESULTS

Hydroponics: Marigold, Tall Fescue, Paddy Rice, and Carrot

The C-14 radioactivity in the culture solution rapidly decreased with time until 8 h after the acetic acid addition, and it continued to decrease slightly to 24 h. At 24 h after the addition, an 87 % reduction in the radioactivity was observed. Radioactivity of a control solution, non-cultured solution, slightly decreased with time. At 24 h after the addition, 18 % of the radioactivity was lost.

Autoradiography images were obtained for the above ground parts of marigold, tall fescue, and paddy rice plants cultured with C-14 labeled solution, but their the sharpness varied. For marigold, there was a clear image for its seed. Specimens and autoradiography images for carrot

seedlings in the pot hydroponics experiment are shown in Fig. II. There was a clear image observed in plants cultured with C-14 labeled solution at the shoot and lower root which was soaked in the culture solution. However, the upper root which was not soaked in the culture solution was not clearly imaged by autoradiography. In the radiotracer experiment using Na-22, a clear autoradiography image was observed for the whole carrot seedling, even in the upper root.



Fig. II Specimens and autoradiography images for carrot seedlings (C-14 addition, left; Na-22 addition, right). Upper image, Shoot; bottom left image; lower root; bottom right image, upper root.

Sand Culture Experiments: Paddy Rice and Radish

Specimen and autoradiography images of paddy rice and radish grown in the sand culture experiments are shown in Fig. III. Both plant types had clear autoradiography images for their roots, but paddy rice shoots had only faint images. The images of radish shoots were clear especially for young leaves. The edible parts of both plant types had good images. The measured radioactivity in an ear of paddy rice was 51 Bq (300 Bq g^{-1} on a fresh weight basis). The radioactivity in the radish edible parts was 377 Bq (180 Bq g^{-1} on a fresh weight basis). These values corresponded to 0.07% and 0.51% of the added radioactivity, respectively.

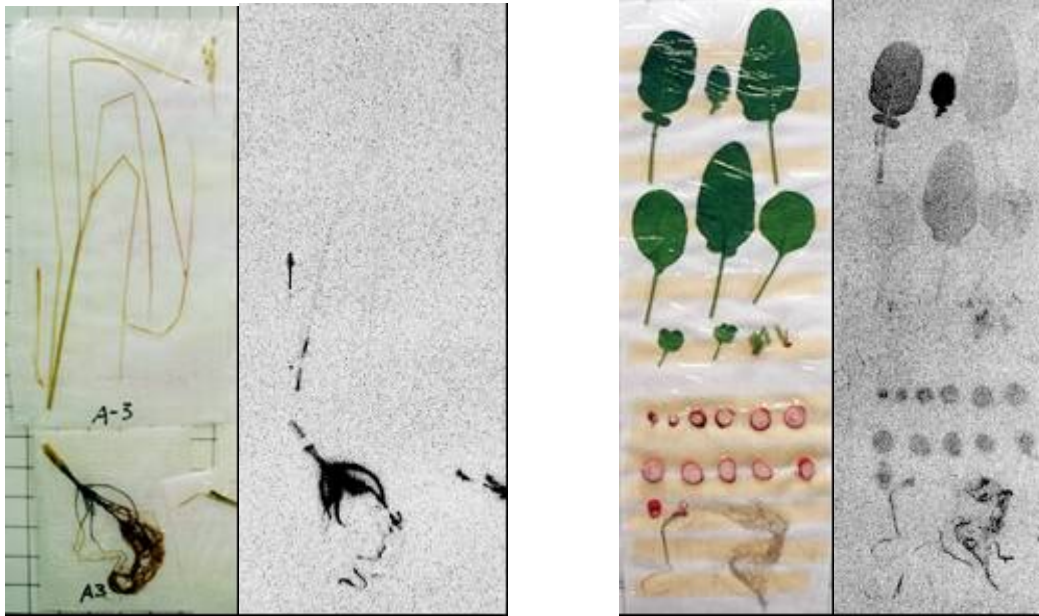


Fig. III Specimens and autoradiography images for paddy rice (left) and radish (right). Upper image, shoot; bottom image, root; upper right corner image, rice edible part; middle image, radish edible part (cross section).

Chamber Experiment: Paddy Rice

Changes in radioactivity of daily and cumulative C-14 in NaOH solution used in the chamber experiment are shown in Fig. IV. The amount of C-14 labeled CO₂ trapped by NaOH solution rapidly increased at first, but quickly decreased 4 days after the addition. Although C-14 labeled CO₂ continued to be trapped by NaOH solution 6 days after the addition, the radioactivity was very low, and cumulative radioactivity did not change considerably. Total C-14 radioactivity as CO₂ trapped by NaOH solution in the chamber was approximately 12 % of added radioactivity 20 days after the addition. In the autoradiography image, the plant did not show radioactivity in the shoot grown under the ambient condition; however, grown inside the chamber, the images showed the plant had radioactivity in the root and young tissues of the shoot such as the emerging leaves.

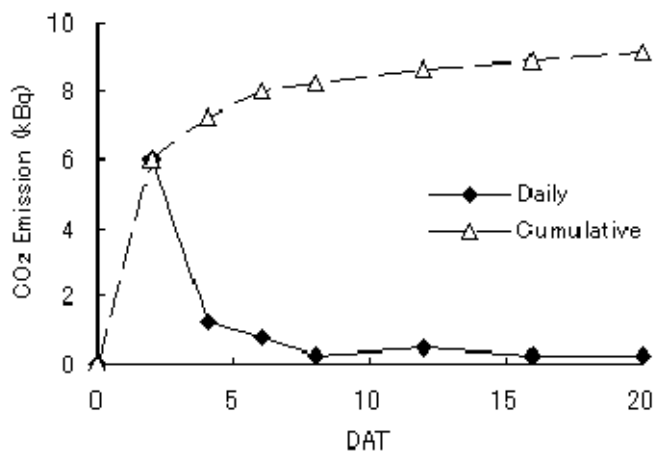


Fig. IV. Changes in activation of daily and cumulative CO₂ in NaOH solution used in the chamber experiment.

DICUSSION

Most C-14 acetic acid was considered to be transformed into inorganic gaseous forms because the sum of radioactivity in the medium and plant parts was very low compared with the added radioactivity. C-14 labeled CO₂ gas was actually released from the culture solution to the atmosphere in the chamber experiment. The C-14 radioactivity was observed in the shoot and root of each plant. However, the C-14 acetic acid absorbed by the plants through their roots was considered to be very small. The increase of radioactivity in the shoot could be caused by absorption of inorganic carbon in gas states that resulted from C-14 acetic acid breakdown in the culture solution by soil microorganisms attached to the root or other minor microorganisms. It was reported that there is an astonishing richness of archaeal diversity present on rice roots well washed with sterilized water [7]. However, the control plant, cultured with C-14 free culture solution and was arranged next to the C-14 labeled plots, did not have radioactivity in any plant parts because of the continuous ventilation in the laboratory. Therefore, though C-14 labeled CO₂ was formed from C-14 acetic acid and emitted from the culture solution to the atmosphere, it was not considered enough for the plant to assimilate into itself during photosynthesis, and it did not exert any appreciable influence on the autoradiography results.

There were strong activities in the lower root part but not in the upper root part as shown by the hydroponics result. This was because the lower root was soaked in the culture solution containing C-14 acetic acid. This indicated that some fraction of C-14 was adsorbed on the root surface and not all the radioactivities were actually caused by C-14 assimilation in the roots. Not much C-14 transferred upward in the plant, suggesting a discrimination of C-14 at the root surface during absorption of various elements from the culture medium. In the radiotracer experiment using Na-22, a clear autoradiography image was observed for the whole carrot seedling, even in the upper root part. The distributions of Na-22 agreed substantially with the

distributions of water in the plant, unlike C-14. One possible reason why C-14 was assimilated to the shoots and not to the upper root was because the plant took up C-14 through the root in inorganic forms such as CO_2 or HCO_3^- with very low concentration. For rice plants, gases generally go up through the aerenchyma system and are emitted to the atmosphere through the stomata. The transfers of gas from the rooting medium to the atmosphere through the rice plant are described by diffusion [8]. C-14 labeled CO_2 may be produced from the breakdown of C-14 acetic acid by microorganisms attached to the root in the culture medium. It may be also broken down and produced by enzymatic activities such as phosphoenolpyruvate carboxylase with the process of plant metabolism in the roots [9]. Inorganic carbon would be taken up by plants through the roots, and some fraction of C-14 would be assimilated to the shoots by photosynthesis. Some studies reported that CO_2 transfer to the rice plant shoots is closely associated with water movement [10, 11]. Others reported that CO_2 transfer to the plant shoots occurs via gaseous forms, and the gasification site is the root cortex where lysigenous intercellular spaces are present [12, 13]. The latter mechanism is more reasonable for the present study.

There would be much organic matter such as carbohydrates including C-14 in the edible parts of rice and radish in the sand culture experiments. Some fraction of C-14 would be transferred and assimilated to the rice in the husk after it was once absorbed by the shoot because there was not so clear autoradiography image of C-14 in the shoot. A relatively longer time for paddy rice would be needed to absorb and assimilate C-14 to the edible part compared with radish. C-14 taken up by rice in the husk and in the edible part of radish may be able to be converted into more complex components such as carbohydrates by photosynthesis and then to be assimilated into the edible part. Transfer of C-14 to edible parts of both plants via roots was generally considered to be very small compared with the added radioactivity.

CONCLUSION

It has been suggested that plants absorbed and assimilated C-14 through their roots. However, the amount of C-14 acetic acid absorbed by plants through the roots was considered to be very small. C-14 acetic acid was broken down into inorganic forms by microorganisms attached to the roots or in the medium or by enzymatic activities involved in the plant metabolism in the roots. Thus, the results indicated that the plants absorbed C-14 through the roots and assimilated it into the shoots or edible parts not because of uptake of C-14 acetic acid but because of uptake of C-14 in inorganic forms such as CO_2 or HCO_3^- with very low concentration. C-14 acetic acid is one of the major components released from ordinary portland cement with is used in solidification for radioactive waste disposal [6], but clarifying its pathways would be difficult because C-14 from radioactive waste would be transformed into inorganic forms in soil. To understand the fate of radioactive nuclides for environmental assessment of waste disposal sites and safety assessment of public health, it is important to monitor the fate of C-14 associated not only with water movement but also with gas transformations even when C-14 is in the soil.

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