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Effect of different Cs concentrations on overall plant growth and Cs distribution in soybean

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ABSTRACT

The absorption and dynamics of Cs in crops can be measured and examined using not only ¹³⁷Cs but also ¹³³Cs, a stable isotope which can be analyzed in a regular laboratory. When ¹³³Cs is used, however, the concentration of added ¹³³Cs must be set higher than that in the soil solution due to the detection sensitivity of inductively coupled plasma optical emission spectrometry (ICP-OES). There is concern that such high Cs concentrations may adversely affect crops and interfere with Cs dynamics. Using soybean as a test product, we first examined the initial growth of soybean cultivated under exposure to solutions with different ¹³³Cs concentrations. In combination with each ¹³³Cs concentration, two potassium (K) concentrations were tested. Results show that at both K concentrations, growth was inhibited under 133 Cs concentrations higher than 1×10^{-3} mol L⁻¹. Soybeans cultivated at 133 Cs concentrations higher than 1×10^{-6} mol L $^{-1}$ can be subject to ICP-OES for measuring 133Cs concentrations in the plant tissue. Next, soybeans cultivated under different 133Cs concentrations throughout growth were examined for Cs distribution in the plants. The distribution patterns of Cs in soybean plants cultivated at ¹³³Cs concentrations equivalent to those found in the soil were almost identical to those in plants cultivated at measurable 133 Cs concentrations (1 \times 10⁻⁶– 1×10^{-4} mol L⁻¹) as measured with ICP-OES. Therefore, in our investigation, ¹³³Cs distribution in soybean plants treated with up to 1×10^{-4} mol L⁻¹ was considered equivalent to that in plants cultivated in the field.

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Introduction

Large amounts of radioactive substances, particularly radiocesium (RCs, 134Cs + 137Cs), were released into the environment as a result of the accident at the Tokyo Electric Power Company Fukushima Daiichi Nuclear Power Plant. Ever since this disaster, there has been concern about the transmission of these radioactive substances from agricultural soils into crops (Yasunari et al., 2011; Zheng et al., 2014). To address these concerns, the dynamics of RCs in the agricultural environment and agricultural crops have been analyzed, and agricultural products have been subjected to monitoring and inspections since the accident (Kinoshita et al., 2011; Nihei et al., 2015). RCs concentrations can be measured with very high accuracy using an NaI (TI) scintillation detector or a germanium semiconductor detector, etc., and the reference value (100 Bq kg⁻¹) for agricultural distribution is equivalent to 3.1×10^{-9} g kg⁻¹ for ¹³⁷Cs. Using the radioactive nuclide ¹³⁷Cs as a physiological tracer in plants enables the measurement of the Cs absorption rate and tissue distribution in crops as well as competitive analysis with potassium (K), an alkali metal (Nobori et al., 2016). However, using ¹³⁷Cs as a tracer requires special permission for the use of a radionuclide and must be performed in a controlled facility that requires additional restrictions unrelated to radioactivity. 133Cs, in contrast, is a stable isotope that exists in nature. The concentration of ¹³³Cs in polluted soil is approximately 7×10^{-3} g kg⁻¹ (Nakai, 2001). Assuming that the partition coefficient K_d (solid phase Cs concentration/Cs solution concentration) is between 269 and 16,637 L kg⁻¹ (Ishikawa et al., 2007), ¹³³Cs concentrations in the soil solution and in crops are estimated to be 1×10^{-9} – 1×10^{-7} mol L⁻¹, respectively (Willey & Martin, 1995). 133Cs concentrations are measured by means of inductively coupled plasma optical emission spectrometry (ICP-OES), ICP mass spectrometry, and the atomic absorption method. The detection limit of Cs with ICP-OES, which can simultaneously perform multi-element analysis and is widely used for the analysis of inorganic elements, is approximately 1 ppm $(10^{-5} \text{ mol L}^{-1})$. Therefore, these techniques can only measure crop ¹³³Cs concentrations that are greater than concentrations



ordinarily present in nature. Because Cs is not an essential element for crops (Hampton et al., 2004), there is concern that growth may be inhibited in crops treated with high Cs concentrations in hydroponic cultures. In an effort to examine the dynamics of absorbed Cs in crops, we should examine whether Cs distribution in crops cultivated under high Cs concentrations is similar to that in samples cultivated at low Cs concentrations and comparable to that cultivated in the soil solution. In this study, we investigated whether high Cs concentrations affected crop growth and Cs distribution by treating plants with solutions containing varying Cs concentrations. Soybean was used as the test crop. In monitoring tests for RCs in Fukushima Prefecture, soybean plants have been shown to contain higher RCs concentrations than other crops (Nihei et al., 2016). It is thus urgent to clarify the factors responsible for this finding.

Materials and methods

Initial growth of soybean plants cultivated in solutions with varying ¹³³Cs concentrations (test I)

The effect of various ¹³³Cs concentrations in cultivation solutions on the initial growth of soybean plants (Glycine max cv. Enrei) was investigated. Soybean was sown in vermiculite. After germination, each sample was transplanted to a test solution (0.3 L) containing a predetermined concentration of ¹³³Cs. The ¹³³Cs concentration in each test solution was set to one of six levels (0, 1×10^{-6} , 1×10^{-5} , 1×10^{-4} , 1×10^{-3} , and 1×10^{-2} mol L⁻¹) by the addition of ¹³³CsCl. For each ¹³³Cs concentration, the K concentration was set to either 3×10^{-5} mol L⁻¹ (hereinafter referred to as low K condition) or 3×10^{-3} mol L⁻¹ (high K condition) by the addition of KCl. Elements other than ¹³³Cs and K were present at concentrations found in a half Hoagland composition (N: 8×10^{-3} mol L⁻¹, P: 1×10^{-3} mol L⁻¹, K: 3×10^{-3} mol L⁻¹, Ca: 2×10^{-3} mol L⁻¹, Mg: 0.5×10^{-3} mol L⁻¹). After transplanting, plants were cultivated for 14 days in a phytotron (16 h light/8 h dark, 28 °C), and the solution was changed once every three days. At the time of harvest, each soybean plant was divided into underground and aboveground parts (cotyledon leaves were removed), and the dry weight was measured after drying at 60 °C for 48 h. These treatments had four replications. To a sample weighing approximately 0.1 g, 10 mL of 60% nitric acid was added. The mixture was heated at 90 °C for 600 min to induce thermal decomposition. After this mixture was diluted to 20 mL with ultrapure water, filtration was conducted with a 0.2-µm membrane filter (Advantec, Japan), and ¹³³Cs, K, Ca, and Mg concentrations were measured using ICP-OES (Optima 7300, PerkinElmer Japan).

Differences in Cs distribution in soybean plants cultivated in solutions with varying 133Cs concentrations (test II)

In an attempt to investigate whether the distribution of Cs in soybean plants was altered in samples cultivated under higher-than-normal ¹³³Cs concentrations, soybeans were grown to maturity under treatment with test solutions containing different ¹³³Cs concentrations, and the Cs concentration and the amount of Cs contained in soybean plants was measured. The concentration of ¹³³Cs in each test solution was set to one of seven levels (0, 1×10^{-9} , 1×10^{-8} , 1×10^{-7} , 1×10^{-6} , 1×10^{-5} , and 1×10^{-4} mol L⁻¹) by the addition of ¹³³CsCl. Because it was assumed that ¹³³Cs levels in soybean plants cultivated at ¹³³Cs concentrations close to those found in the soil solution (1×10^{-9} to 1×10^{-7} mol L⁻¹, Willey & Martin, 1995) could not be measured by ICP-OES, 100 Bg L^{-1} (2.3 × 10⁻¹³ mol L^{-1}) of ¹³⁷Cs was added to each solution as a tracer to measure the amount of Cs in each part of each soybean plant. Since the added quantity of ¹³⁷Cs was extremely small with respect to that of ¹³³Cs, the total amount of Cs added can be approximated as the amount of ¹³³Cs. Because the molecular weight of Cs is 130 or higher, ¹³⁷Cs and ¹³³Cs can be considered to exhibit the same chemical dynamics in the samples (Slat et al., 2004). Elements other than Cs were present in the solution at the concentrations found in a half Hoagland composition $(N: 8 \times 10^{-3} \text{ mol L}^{-1}, P: 1 \times 10^{-3} \text{ mol L}^{-1}, K: 3 \times 10^{-3} \text{ mol L}^{-1},$ Ca: 2×10^{-3} mol L⁻¹, and Mg: 0.5×10^{-3} mol L⁻¹). Soybean plants, after germination, were transplanted to a hydroponic solution (2.5 L) containing a predetermined element concentration. The hydroponic liquid was changed once a week. These treatments had three replications. In the latter part of the growing phase, an appropriate quantity of the hydroponic solution was added according to the transpiration rate of each soybean plant. Soybean plants were cultivated for 83 days within the phytotron (8 h light /16 h dark, 28 °C) in a radioisotope-controlled area until harvesting. To equalize conditions inside the phytotron (such as light exposure), the soybean containers were rotated weekly. Each cultivated soybean plant was divided into its parts (stem, leaf, petiole, pod, and seed), and the amount of ¹³⁷Cs in each part was measured with via Nal scintillation detector (ARC-300, Aloka, Japan). The dry weight was measured after drying at 60 °C for two days, and the ¹³⁷Cs distribution ratio and concentration in each part were calculated.

Results and discussion

Initial growth of soybean plants cultivated in solutions with varying 133Cs concentrations (test I)

Figure 1 shows the dry weights of the aboveground and underground parts of soybean plants grown in solutions

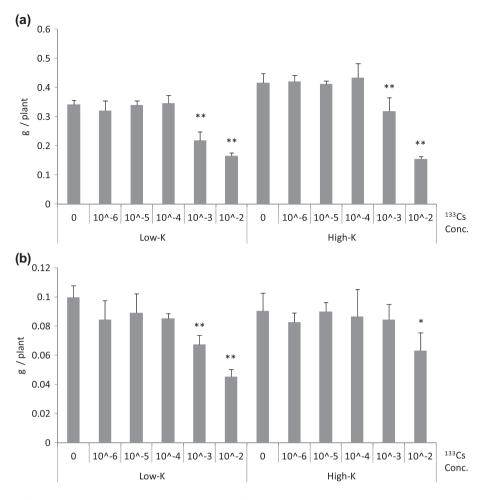


Figure 1. Dry weight of the aboveground and underground parts of soybean plants grown in solutions containing varying ¹³³Cs and K concentrations.

Notes: S and R represent aboveground parts and underground parts, respectively. Data are shown as mean \pm standard deviation. Asterisks (**p < 0.01, *p < 0.05) indicate a notable difference from 0 μ M ¹³³Cs based on Dunnett's ANOVA test.

containing different ¹³³Cs concentrations. Soybean plants grown at ^{133}Cs concentrations between 0 and 1 \times 10 $^{-4}$ mol L^{-1} had equivalent dry weights at both K concentrations. In soybean plants grown in ¹³³Cs concentrations higher than 1×10^{-3} mol L⁻¹, the dry weights decreased by 35% under low K conditions and by 21% under high K conditions. Figure 2 shows ¹³³Cs and K concentrations in the aboveground and underground parts of soybean plants. Except for the underground parts grown at 1×10^{-2} mol L⁻¹ of ¹³³Cs under low K conditions, the ¹³³Cs concentration in each plant part increased as the ¹³³Cs concentration increased, regardless of K condition. The K concentration, meanwhile, decreased at 1×10^{-2} mol L⁻¹ of ¹³³Cs in the underground parts under low K conditions, at more than 1×10^{-5} mol L⁻¹ of ¹³³Cs in the aboveground parts under high K conditions, and at more than 1×10^{-3} mol L⁻¹ of ¹³³Cs in the underground parts under high K conditions. The Cs⁺ ion has similar chemical properties to the K⁺ ion and competes with the K⁺ ion for cation binding sites in proteins (Hampton et al., 2004). Unfortunately, the Cs⁺ ion does not behave identically to the K⁺ ion and inhibits the activity of many K-activated enzymes (Avery, 1995). The Cs⁺ ion also inhibits the inward-rectifying K channels in the plasma membranes of plant cells (White, 1997). Excessive Cs in the rhizosphere could therefore induce K starvation in plants (Hampton et al., 2004). Growth inhibition by ¹³³Cs in hydroponic solutions has been reported at concentrations of 1×10^{-5} mol L⁻¹ in rice (Hasegawa, 2012) and 5×10^{-5} mol L⁻¹ in *Arabidopsis thaliana* (Sheahan et al., 1993). In the present study, however, growth inhibition was observed only at concentrations higher than those reported previously (1 \times 10⁻³ mol L⁻¹). This difference might be a result of difference test crops. In addition, in our study, each test solution contained not only ¹³³Cs but also K and other nutritional elements, whereas those used in previous reports (Hasegawa, 2012; Sheahan et al., 1993) contained only ¹³³Cs as a single salt. We speculate that these differences were among the factors responsible for differing in results. Futher, it has been reported that Cs is analogous to K, in that the growth-inhibiting effect of

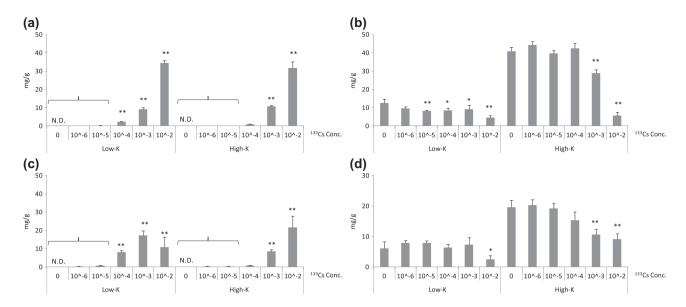


Figure 2. 133 Cs and K concentrations in the aboveground and underground parts of soybean plants grown in solutions containing varying 133 Cs and K concentrations. Data are shown as mean \pm standard deviation. (a) 137 Cs concentrations in aboveground, (b) K concentrations in aboveground, (c) 137 Cs concentrations in underground, and (d) K concentrations in underground.

Notes: Asterisks (**p < 0.01, *p < 0.05) indicate a notable difference from 10–6 μ M ¹³³Cs in (a) and (c), and 0 μ M ¹³³Cs in (b) and (d) based on Dunnett's ANOVA test.

Cs is mitigated by the presence of K and that the K concentration in crops affects Cs dynamics (Zhu & Smolders, 2000). With the exception of 1×10^{-2} mol L⁻¹ of ¹³³Cs, which inhibited growth due to the significant elevation of Cs concentration, ¹³³Cs concentrations in the high K conditions were lower than those in the low K conditions. The growth inhibition that resulted from a ¹³³Cs concentration of 1×10^{-3} mol L⁻¹ was also low in the high K conditions. In the aboveground plant parts, however, there was no difference in Cs concentration between the low and high K conditions. Further study is necessary to investigate detailed Cs dynamics. Moreover, accumulated ¹³³Cs in the aboveground parts of the crop at 1×10^{-6} mol L⁻¹ was approximately 4×10^{-2} g kg⁻¹. Under our decomposition conditions (approximately 0.1 g of soybean plant in 20 mL of decomposition solution), the ¹³³Cs concentration in the decomposition solution was approximately 1 ppm, and this concentration could be measured using ICP-OES. It should be considered that soybeans grown at ¹³³Cs concentrations between $1\times 10^{-6}\, mol\, L^{-1}$ and $1\times 10^{-4}\, mol\, L^{-1}$ do not experience inhibited growth, and can be measured with ICP-OES.

Differences in Cs distribution in soybean plants cultivated in solutions with varying ¹³³Cs concentrations (test II)

In order to compare Cs concentration of each soybean part, soybeans were cultivated under different ^{133}Cs concentrations (0–1 \times 10⁻⁴ mol L⁻¹ solution) and at high K conditions (3 \times 10⁻³ mol L⁻¹) until harvest. After the

disaster, farmers who cultivated soybeans in Fukushima Prefecture were recommended to fertilize potassium because it became clear that potassium fertilization was effective for reducing radiocesium concentrations in soybeans. As such, there are few potassium-deficient fields in Fukushima Prefecture. We considered that it is important to analyze Cs distribution in soybeans under the K conditions which soybeans are cultivated in Fukushima Prefecture, and test II was conducted under the high K condition. Figure 3 shows the ¹³⁷Cs concentrations in the various aboveground plant parts. The concentration of 137 Cs was 27–37 Bg kg $^{-1}$ in the stem, 24–33 Bg kg $^{-1}$ in the leaf, 26-37 Bq kg⁻¹ in the petiole, 13-21 Bq kg⁻¹ in the pod and 13–17 Bg kg⁻¹ in the seed. These concentrations were not significantly affected by increased ¹³³Cs concentrations in the solution. Figure 4 shows the weight and distribution ratio of ¹³⁷Cs for each part of the soybean plant. The weight distribution for each part in soybean plants grown at various Cs concentrations was 6-8% in the stem, 18-20% in the leaf, 2-3% in the petiole, 18-24% in the pod and 47–54% in the seed. The distribution rate of ¹³⁷Cs concentration was 9–15% in the stem, 24–32% in the leaf, 3–6% in the petiole, 17–19% in the pod and 36–44% in the seed. These ratios were not significantly affected by increased ¹³³Cs concentrations in the solution. Under our experimental K concentration, absorbed Cs distributions in soybean plant parts grown at high ¹³³Cs concentrations $(1 \times 10^{-6} - 1 \times 10^{-4} \text{ mol L}^{-1})$ which can be measured by ICP-MS were similar to that in soybeans grown at low ¹³³Cs concentrations (1 \times 10⁻⁹–1 \times 10⁻⁷ mol L⁻¹), close to those found in the soil solution.

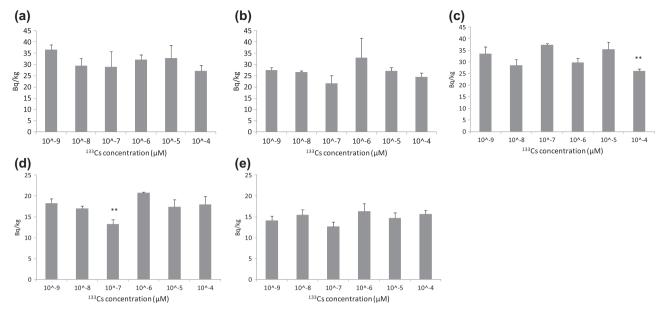


Figure 3. 137 Cs concentrations in the various parts of soybean plants treated with various 133 Cs concentrations in a hydroponic solution. (a)stem, (b)leaf, (c)petiole, (d)pod, (e)seed. Notes: Asterisk (**p < 0.01) indicates a notable difference from $10^{-9} \, \mu M$ 133 Cs based on Dunnett's ANOVA test.

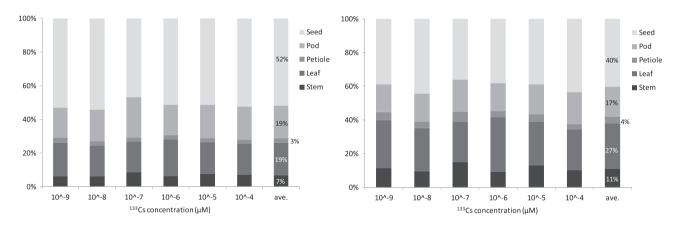


Figure 4. ¹³⁷Cs distribution rates in the various parts of soybean plants treated with various ¹³³Cs concentrations in a hydroponic solution. Left; distribution of weight, right; distribution of ¹³⁷Cs concentration.

These results lead to the conclusion that the distribution of Cs in soybean plants can be analyzed without the use of RI-controlled facilities under high ^{133}Cs concentration condition (1 \times 10 $^{-6}$ –1 \times 10 $^{-4}$ mol L $^{-1}$) which do not inhibit soybean plant growth and are measurable with ICP-MS.

Disclosure statement

No potential conflict of interest was reported by the authors.

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