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 133Cs concentrations higher than 1 × 10⁻³ mol L⁻¹. Soybeans cultivated at 133Cs 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 133Cs concentrations (1 × 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.

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 137Cs 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 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 $^{133}$Cs concentrations (test I)

The effect of various $^{133}$Cs 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 $^{133}$Cs. The $^{133}$Cs concentration in each test solution was set to one of six levels ($0, 1 \times 10^{-6}, 1 \times 10^{-5}, 1 \times 10^{-4}, 1 \times 10^{-3}, \text{and } 1 \times 10^{-2}$ mol L$^{-1}$) by the addition of $^{133}$CsCl. For each $^{133}$Cs concentration, the K concentration was set to either $3 \times 10^{-5}$ mol L$^{-1}$ (herein, after referred to as low K condition) or $3 \times 10^{-3}$ mol L$^{-1}$ (high K condition) by the addition of KCl. Elements other than $^{133}$Cs and K were present at concentrations found in a half Hoagland composition (N: $8 \times 10^{-3}$ mol L$^{-1}$, P: $1 \times 10^{-3}$ mol L$^{-1}$, K: $3 \times 10^{-3}$ mol L$^{-1}$, Ca: $2 \times 10^{-3}$ mol L$^{-1}$, Mg: $0.5 \times 10^{-3}$ 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 a week. These treatments had three replications. In the latter part of the growing phase, an appropriate quantity of the hydroponic liquid containing absorbed Cs was added to each sample as a tracer to measure the amount of Cs in each part of each soybean plant. In this study, we investigated the distribution of Cs in soybean plants cultivated under higher Cs concentrations. Soybean plants were grown to maturity under treatment with test solutions containing different $^{133}$Cs concentrations, and the Cs concentration and the amount of Cs contained in soybean plants was measured. The concentration of $^{131}$Cs in each test solution was set to one of seven levels ($0, 1 \times 10^{-9}, 1 \times 10^{-8}, 1 \times 10^{-7}, 1 \times 10^{-6}, 1 \times 10^{-5}, \text{and } 1 \times 10^{-4}$ mol L$^{-1}$) by the addition of $^{131}$CsCl. Because it was assumed that $^{133}$Cs levels in soybean plants cultivated at $^{133}$Cs concentrations close to those found in the soil solution ($1 \times 10^{-9} \text{ to } 1 \times 10^{-7}$ mol L$^{-1}$, Willey & Martin, 1995) could not be measured by ICP-OES, 100 Bq L$^{-1}$ ($2.3 \times 10^{-13}$ mol L$^{-1}$) of $^{133}$Cs 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 $^{137}$Cs was extremely small with respect to that of $^{133}$Cs, the total amount of Cs added can be approximated as the amount of $^{133}$Cs. Because the molecular weight of Cs is 130 or higher, $^{137}$Cs and $^{133}$Cs 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 concentrations found in a half Hoagland composition (N: $8 \times 10^{-3}$ mol L$^{-1}$, P: $1 \times 10^{-3}$ mol L$^{-1}$, K: $3 \times 10^{-3}$ mol L$^{-1}$, Ca: $2 \times 10^{-3}$ mol L$^{-1}$, and Mg: $0.5 \times 10^{-3}$ 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 $^{137}$Cs in each part was measured with NaI scintillation detector (ARC-300, Aoloka, Japan). The dry weight was measured after drying at 60 °C for two days, and the $^{137}$Cs distribution ratio and concentration in each part were calculated.

Results and discussion

Initial growth of soybean plants cultivated in solutions with varying $^{133}$Cs 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 $^{133}$Cs concentrations, soybeans were grown to maturity under treatment with test solutions containing different $^{133}$Cs concentrations, and the Cs concentration and the amount of Cs contained in soybean plants was measured. The concentration of $^{131}$Cs in each test solution was set to one of seven levels ($0, 1 \times 10^{-9}, 1 \times 10^{-8}, 1 \times 10^{-7}, 1 \times 10^{-6}, 1 \times 10^{-5}, \text{and } 1 \times 10^{-4}$ mol L$^{-1}$) by the addition of $^{131}$CsCl. Because it was assumed that $^{133}$Cs levels in soybean plants cultivated at $^{133}$Cs concentrations close to those found in the soil solution ($1 \times 10^{-9} \text{ to } 1 \times 10^{-7}$ mol L$^{-1}$, Willey & Martin, 1995) could not be measured by ICP-OES, 100 Bq L$^{-1}$ ($2.3 \times 10^{-13}$ mol L$^{-1}$) of $^{133}$Cs 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 $^{137}$Cs was extremely small with respect to that of $^{133}$Cs, the total amount of Cs added can be approximated as the amount of $^{133}$Cs. Because the molecular weight of Cs is 130 or higher, $^{137}$Cs and $^{133}$Cs 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 concentrations found in a half Hoagland composition (N: $8 \times 10^{-3}$ mol L$^{-1}$, P: $1 \times 10^{-3}$ mol L$^{-1}$, K: $3 \times 10^{-3}$ mol L$^{-1}$, Ca: $2 \times 10^{-3}$ mol L$^{-1}$, and Mg: $0.5 \times 10^{-3}$ 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 $^{137}$Cs in each part was measured with NaI scintillation detector (ARC-300, Aoloka, Japan). The dry weight was measured after drying at 60 °C for two days, and the $^{137}$Cs distribution ratio and concentration in each part were calculated.

Results and discussion

Initial growth of soybean plants cultivated in solutions with varying $^{133}$Cs concentrations (test II)

Figure 1 shows the dry weights of the aboveground and underground parts of soybean plants grown in solutions
containing different $^{133}$Cs 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 $^{133}$Cs concentrations higher than $1 \times 10^{-3}$ mol L$^{-1}$, the dry weights decreased by 35% under low K conditions and by 21% under high K conditions. Figure 2 shows $^{133}$Cs and K concentrations in the aboveground and underground parts of soybean plants. Except for the underground parts grown at $1 \times 10^{-2}$ mol L$^{-1}$ of $^{133}$Cs under low K conditions, the $^{133}$Cs concentration in each plant part increased as the $^{133}$Cs concentration increased, regardless of K condition. The K concentration, meanwhile, decreased at $1 \times 10^{-2}$ mol L$^{-1}$ of $^{133}$Cs in the underground parts under low K conditions, at more than $1 \times 10^{-5}$ mol L$^{-1}$ of $^{133}$Cs in the aboveground parts under high K conditions, and at more than $1 \times 10^{-3}$ mol L$^{-1}$ of $^{133}$Cs 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 $^{133}$Cs in hydroponic solutions has been reported at concentrations of $1 \times 10^{-5}$ mol L$^{-1}$ in rice (Hasegawa, 2012) and $5 \times 10^{-5}$ 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 $^{133}$Cs but also K and other nutritional elements, whereas those used in previous reports (Hasegawa, 2012; Sheahan et al., 1993) contained only $^{133}$Cs as a single salt. We speculate that these differences were among the factors responsible for differing in results. Further, it has been reported that Cs is analogous to K, in that the growth-inhibiting effect of

Figure 1. Dry weight of the aboveground and underground parts of soybean plants grown in solutions containing varying $^{133}$Cs and K concentrations.
Notes: S and R represent aboveground parts and underground parts, respectively. Data are shown as mean ± standard deviation. Asterisks (**p < 0.01, *p < 0.05) indicate a notable difference from 0 μM $^{133}$Cs based on Dunnett’s ANOVA test.
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 137Cs was 27–37 Bq kg⁻¹ in the stem, 24–33 Bq kg⁻¹ in the leaf, 26–37 Bq kg⁻¹ in the petiole, 13–21 Bq kg⁻¹ in the pod and 13–17 Bq 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 47–54% 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 × 10⁻⁶–1 × 10⁻⁴ mol L⁻¹) which can be measured by ICP-MS were similar to that in soybeans grown at low 133Cs concentrations (1 × 10⁻⁹–1 × 10⁻⁷ mol L⁻¹), close to those found in the soil solution.

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 × 10⁻⁶ mol L⁻¹ and 1 × 10⁻² 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 133Cs concentrations (0–1 × 10⁻⁴ mol L⁻¹ solution) and at high K conditions (3 × 10⁻³ mol L⁻¹) until harvest. After the
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 133Cs concentration condition (1 × 10⁻⁶–1 × 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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