Article

The Concentration Distributions of Cs in Soybean Seeds

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Radioactive materials, primarily radiocaesium (^{134}Cs + ^{137}Cs), were released into the environment by the Fukushima Dai-ichi Nuclear Power Plant accident in March 2011. The proportion of the soybean plants that had an activity concentration of radiocaesium over 100 Bq/kg was higher than that of other crops. To examine the reason why the activity concentration of radiocaesium in soybeans was higher, the concentration distributions of Cs in soybean seeds were analyzed using two methods, X-ray fluorescence microscopy and radioluminography with ^{137}Cs . Moreover, the Cs concentration of each organ and the ratio of absorbed Cs to seeds in matured soybeans were examined. The results of the two experiments show that Cs was uniformly distributed in the soybean seeds, as was potassium, both of which likely accumulated in the cotyledon. In addition, approximately 40% of absorbed Cs was accumulated in the soybean seeds. Conversely, it is reported that rice accumulates Cs only in part of its grain and that the accumulation in the grain is lower than that in soybeans. It has been suggested that soybean seeds have a large capacity for Cs accumulation, and this is one of the reasons why the activity concentrations of radiocaesium in soybeans are higher than that in other crops.

Key Words: radiocaesium, radioluminography, soybean, X-ray fluorescence microscopy

1. Introduction

Radioactive materials were released by the Fukushima Dai-ichi Nuclear Power Plant accident on March 11, 2011. These radioactive materials dominated by radiocaesium (^{134}Cs and ^{137}Cs) fell on various agricultural lands in Fukushima and the neighboring prefectures.^{1,2)} To assess the safety of agricultural products, the Nuclear Emergency Response Headquarters has been conducting emergency environmental radiation monitoring of agri-

cultural and fishery products (hereafter, referred to as “monitoring inspections”).³⁾

The monitoring inspections indicate that the ratio of radiocaesium within soybean plants exceeds 100 Bq kg^{−1} wet plant, the limit for radiocaesium in general foods, and is high compared to other crops.⁴⁾ Because the cultivation area of soybean plants in the Fukushima Prefecture is the second largest after rice, the analysis of caesium (Cs) absorption in soybean plants is particularly important. Even though there are some reports of Cs absorption by soybean

plants,⁴⁾ it is not clear why the concentrations of Cs in soybean seeds are higher than those in other crops. Because the monitoring inspections measured the edible parts of the crop, the seed in the case of soybeans, the distributions of Cs in soybean seeds needs to be explained.

The isotopes of Cs are composed of stable caesium (^{133}Cs), which is included in minerals naturally, and radiocaesium, which was released artificially. It has been reported that approximately 7 mg kg^{-1} of Cs exists in Japanese soil.⁵⁾ Conversely, assuming that the polluted soil has 5000 Bq kg^{-1} of radiocaesium, this would correspond to approximately $1.6 \times 10^{-6}\text{ mg kg}^{-1}$. ^{133}Cs and ^{137}Cs can be thought of as having the same chemical behavior. Accordingly, ^{133}Cs has been used in various examinations to predict ^{137}Cs behavior.⁶⁾

To map these elements at the microstructural level, several methods have been developed, such as X-ray fluorescence microscopy,⁷⁾ scanning electron microscopy with energy dispersive X-ray spectrometry,^{8,9)} and autoradiography. X-ray techniques can detect multiple elements simultaneously and can reveal the chemical form of an element. An analytic X-ray microscope has a thin glass tube that focuses an X-ray beam, enabling the visualization of elements at resolutions of $10\text{--}100\text{ }\mu\text{m}$. In addition to this high resolution, biological specimens can be analyzed under normal pressures, without requiring chemical or drying pretreatment of the sample. This system can be used without a radiation-controlled area. However, the detection limit is approximately 0.1%. Therefore, it is necessary to cultivate a crop with a culture including high Cs levels to analyze the Cs distribution in seeds with X-ray fluorescence microscopy. To date, there are no reports of analyzed Cs distributions in seeds with X-ray fluorescence microscopy. Consequently, it is unclear how much Cs is necessary when cultivating a crop. Furthermore, the Cs concentration in a seed that can be detected

by X-ray fluorescence microscopy is very high compared with that of an actual seed. To confirm the Cs distribution of a seed cultivated at a high concentration, it is necessary to make comparisons with seeds cultivated at low concentrations using radiocaesium.

The application of radioactive materials as tracers is advantageous from the point of view of sensitivity, and autoradiography is generally performed to obtain radioactivity distribution images. In the case of autoradiography, and imaging plate (IP) based on radioluminography and which has high sensitivity, high quantitative performance and a simple protocol, is widely used.¹¹⁾ The detection limit depends on the exposure time and the type of radioactive material, for example, 7.4 kBq g^{-1} (45 ng g^{-1} as ^{14}C) requires a 60 min exposure to the IP.¹¹⁾ In this case, the resolution exceeds $10\text{ }\mu\text{m}$ per pixel due to the performance of currently available image readers.¹⁰⁾ However, a radiation-controlled area is necessary to use such radioactive material.

The goal of this study is to determine why the activity concentrations of radiocaesium in soybeans are higher than that in other crops. Two different methods are used to explore the Cs distribution in a soybean seed, X-ray fluorescence microscopy targeting ^{133}Cs and radioluminography targeting ^{137}Cs .

2. Materials and Methods

2.1 Analysis of Cs with X-ray fluorescent microscopy (Experiment A)

To analyze the Cs distribution in a soybean seed with X-ray microscopy, soybeans were cultivated under conditions where the Cs concentration was varied. Germinated soybean (*Glycine max* cv. Enrei) seeds were cultivated in 500 mL plastic containers with a mineral nutrient medium adjusted to a pH of 6.0 containing ^{133}Cs at concentrations of 0.01 mM, 0.1 mM, and 1 mM in the form of $^{133}\text{CsCl}$ during the developmental stages. The mineral nutrient medium contained 0.28 mM MgSO_4 , 0.6 mM

KNO₃, 0.084 mM KCl, 0.13 mM KH₂PO₄, 0.24 mM Ca(NO₃)₂, 2 μ M Fe-EDTA, 4.5 μ M KI, 28 μ M MnCl₂, 19 μ M H₃BO₃, 2.3 μ M ZnSO₄, 0.5 μ M CuSO₄, and 0.003 μ M Na₂MoO₄, as previously described.¹²⁾ Chemicals were purchased from Wako Pure Chemicals or Nacalai tesque, unless otherwise stated. Soybeans were kept in a culture room at 28°C, with a 16-h photoperiod. After dividing the matured soybean seeds into two pieces, their distributions of Cs, Potassium (K), and Calcium (Ca) were analyzed using a X-ray fluorescent microscope (XGT-5200WR, Horiba, Kyoto, Japan) under the following conditions: a glass tube diameter of 1200 μ m, a tube voltage of 15 kV, and an acquisition time of 1500 s/frame.

2.2 Analysis of Cs with radioluminography (Experiment B)

The Cs concentration used in Experiment A is much higher than that measured in the contaminated area. To analyze the Cs distribution in a soybean seed at low Cs concentration, germinated soybeans were cultured in 3000 mL plastic containers with a mineral nutrient medium adjusted to a pH of 6.0 containing 100 Bq L⁻¹ (2.3×10^{-7} μ M) of ¹³⁷Cs (Eckert & Ziegler) in addition to 0.01 μ M of ¹³³Cs as a carrier. Both ¹³⁷Cs and ¹³³Cs were introduced into the medium as CsCl. It has been reported that approximately 7 mg/kg of ¹³³Cs exists in Japanese soil,⁵⁾ and its distribution coefficient (K_d) is 269–16,637 L kg⁻¹.¹³⁾ Therefore, the ¹³³Cs concentration in the soil solution is likely 0.003–0.2 μ M. The additional ¹³³CsCl concentration was set to 0.01 μ M. The nutrient medium contained 0.5 mM MgSO₄, 3 mM KNO₃, 0.025 mM KCl, 1 mM KH₄H₂PO₄, 2 mM Ca(NO₃)₂, 2 μ M Fe-EDTA, 1 μ M MnSO₄, 12.5 μ M H₃BO₃, 1 μ M ZnSO₄, 0.25 μ M CuSO₄, and 0.003 μ M Na₂MoO₄, as previously described.¹¹⁾ The soybeans were kept in a culture room at 28°C, with a 16-h photoperiod for 83 d. The solution was renewed twice

a week. The experiment was performed with three repetitions. To analyze the Cs distribution in the soybean seeds, the harvested seeds were sectioned using a Multi-Purpose Cryosection Preparation Kit (Section-Lab, Japan), as previously described.^{14–16)} The sections were prepared and put on an IP (BAS IP TR, GE Healthcare, UK), and the IP was exposed in a freezer at –80°C for 6 d. Images obtained by the IP were scanned with an image analyzer (FLA5000, Fujifilm, Japan) at a resolution of 10 μ m. In addition, the K, Ca, and Mg distributions of the sections were measured using a Scanning Electron Microscope (SU3500, Hitachi, Japan) equipped with energy dispersive X-ray analyzer (EMAX X-Max^N 50 mm², Horiba, Japan).

In addition, to examine the activity concentration of ¹³⁷Cs in each organ and the ratio of the absorbed ¹³⁷Cs to seeds in matured soybeans, the harvested plants were rinsed under running water and divided into different organs (i.e., root, stem, leaf, petiole, pod, and seed). After drying these organs at 60°C for 48 h, the activity concentration of ¹³⁷Cs was measured with a NaI scintillation counter (ARC-300, Aloka, Japan). The amount of ¹³⁷Cs were estimated with the activity concentration of ¹³⁷Cs and the dry weight in each organ.

3. Results

¹³³Cs was detected by X-ray fluorescent microscopy in soybean seeds grown in a 1 mM ¹³³CsCl solution but not in those grown in 0.01 mM or 0.1 mM ¹³³CsCl solutions. The cross-sectional distributions of ¹³³Cs in soybean seeds were found to be uniform when grown in the 1 mM ¹³³CsCl solution. The distributions of K and Ca were also uniform in the seeds, irrespective of the ¹³³Cs concentration (Fig. 1).

Fig. 2 displays the autoradiography showing that the distribution of ¹³⁷Cs in seeds cultured in low Cs concentrations was uniform, as seen in Experiment A. Fig. 3 shows an SEM-EDX image of a soybean

Fig. 1 The cross-sectional distributions of Cs, K, and Ca in soybean seeds analyzed using an X-ray fluorescent microscope: (A) bright field image; (B) distribution of Cs; (C) distribution of K; (D) distribution of Ca. The caesium chloride concentrations in the cultivated solutions were (1) 0 mM; (2) 0.01 mM; (3) 0.1 mM; (4); and (5) 1 mM. Scale bar=5 mm. After dividing the matured soybean seeds into two pieces, their distributions of Cs, K, and Ca were analyzed using a X-ray fluorescent microscope (XGT-5200WR, Horiba, Kyoto, Japan) (Color online).

Fig. 2 The cross-sectional distributions of radiocaesium (^{137}Cs) in soybean seeds analyzed using an imaging plate: (A) photograph of the seed section, upper: vertical section, lower: horizontal section; and (B) autoradiograph of (A) showing the localization of ^{137}Cs . Scale bar=5 mm. The sectioned seeds were put on an IP, and the IP was exposed in a freezer at -80°C for 6 d. Images obtained by the IP were scanned with an image analyzer (FLA5000, Fujifilm, Japan) at a resolution of $10\ \mu\text{m}$.

seed. The distribution of K and Mg inside the seeds was also uniform; however, Ca was moderately localized in the seed. It has been reported that calcium oxalate crystals develop in soybean seeds.¹⁷⁾

The activity concentration of ^{137}Cs in each organ was measured in the matured soybean plant. The concentration increased in the following order: seed<pod<leaf<stem<petiole<root (Fig. 4a). The amount of ^{137}Cs increased in the following order: petiole<root<stem<pod<leaf<seed (Fig. 4b). The weight ratios of organs in the matured soybean were 52% for the seeds, 18% for the pods, 2% for the petioles, 18% for the leaves, 6% for the stems, and 4% for the roots. Conversely, the activity concentration ratios of organs in the matured soybean were 42% for the seeds, 16% for the pods, 4% for the petioles, 24% for the leaves, 9% for the stems, 6% for the roots (Fig. 5).

4. Discussion

Soybean seeds do not develop their albumen;

Fig. 3 The cross-sectional distributions of K, Mg, and Ca in a soybean seed analyzed using an SEM-EDX: (A) bright field image; (B) distribution of K; (C) distribution of Mg; and (D) distribution of Ca. Scale bar=5 mm. The K, Ca, and Mg distributions of the sections were measured using a Scanning Electron Microscope (SU3500, Hitachi, Japan) equipped with energy dispersive X-ray analyzer (EMAX X-Max^N 50 mm², Horiba, Japan).

therefore, they are called exalbuminous seeds. The cotyledon capacity occupies the largest part of soybean seeds.¹⁸⁾ Figs. 2 and 4 show that the concentration distributions of K in soybean seeds are uniform and that potassium ion within the soybean seeds accumulates in the cotyledon. In addition, from Figs. 2 and 3, it can be seen that the concentration distributions of Cs in soybean seeds are also uniform, similar to that of K. The chemical behavior of Cs can be expected to be similar to that of K because they are both alkali metal elements and have similar physico-chemical properties.¹⁹⁾ Therefore, it is assumed that Cs is also accumulated in the cotyledon.

However, the monitoring inspections indicated that the ratio of radiocaesium proportions within soybeans exceeding 100 Bq kg⁻¹ was high compared to that of rice. Moreover, the K concentrations in soybean seeds are approximately five times higher

than that of rice grains.²⁰⁾ Rice grains accumulate K in the embryo. The K concentrations of the seeds or grains depend on how much K is accumulated in these organs. If the K concentrations in the cultivated area is similar to that accumulated in seeds and grains, it is likely that K concentrations are due to the capacity of seeds or grains to accumulate K. The embryo capacity is only a small part of the grain, whereas the cotyledon capacity is the dominant capacity of a soybean seed. Therefore, the K concentrations in soybean seeds will be higher than that in rice grains. Hirose et al.²¹⁾ reported that the concentration distributions of ¹³⁷Cs in rice grains are localized and similar to that of K. The different distributions of ¹³⁷Cs for rice grains and soybean seeds appear to be derived from their seed storage tissues. The monitoring inspections measured the edible parts of the crop,⁴⁾ i.e., seeds and grains for soybean

Fig. 4 (A) The activity concentration of ^{137}Cs and (B) the amount of ^{137}Cs in each organ. Soybeans were cultured with a nutrient medium containing 100 Bq L^{-1} of ^{137}Cs in addition to $0.01 \mu\text{M}$ of ^{133}Cs . The soybeans were kept at 28°C , with a 16-h photoperiod for 83 d. After drying these organs at 60°C for 48 h, the activity concentration of ^{137}Cs was measured with a NaI scintillation counter (ARC-300, Aloka, Japan). The amount of ^{137}Cs were estimated with the activity concentration of ^{137}Cs and the dry weight in each organ.

Fig. 5 The weight and the ^{137}Cs activity concentration ratios of organs in the matured soybean.

and rice, respectively. The results suggested that the large capacity of Cs accumulation in soybean seeds is one of the reasons why the activity concentration

of radiocaesium in soybeans in the monitoring inspections was higher than that of rice.

On the other hand, soybean seeds accumulate approximately 40% of the entire amount of Cs that the soybean plants absorb (Fig. 5). While rice grains only accumulate 20% of the entire amount absorbed.²²⁾ It is not clear whether the amount of Cs that the soybean plants absorb is larger than that of rice; however, it has been suggested that soybean plants translocate absorbed Cs to its seeds more easily than do other crops.

The soybean cultivation area in the Fukushima Prefecture is the second largest cultivation area after rice. Soybean seeds are very efficient in accumulating K; unfortunately, this ability includes the accumulation of radiocaesium. Therefore, to assist the recovery and revitalization of agriculture in this area, it is important to develop agricultural techniques that inhibit Cs accumulation in soybean plants. One technique is the use of K fertilizer, which has been used in particularly contaminated areas. Further studies will be needed to develop more efficient techniques, such as the examination of the mechanism of Cs accumulation in soybean seeds and the improvement of soybean breeds able to alleviate the absorption of radiocaesium.

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要 旨

ダイズ子実中のセシウムの濃度分布

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他の作物よりもダイズ子実中の放射性Cs濃度は高いがその理由は明らかにされていない。本論文では子実内のCs分布に着目し、¹³³Csを用いたX線蛍光顕微鏡と、¹³⁷Csを用いたオートラジオグラフィで観察するとともに、成熟期のダイズ体内のCs分布も検討した。CsはKと同様に均一に分布し、吸収したCsの約4割が子実に蓄積した。ダイズ子実は他の作物よりCsを蓄積する割合が大きく、吸収したCsを子実に多く蓄えることが、放射性Cs濃度が高くなる要因の一つと考えられた。