

Chapter 3

Cesium Translocation in Rice



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Abstract To breed a low Cs rice variety, it is important to clarify the mechanism of Cs transport in a plant. In the present report, we found a difference in Cs distribution in rice cultivars using a ^{137}Cs tracer experiment. In addition, the difference was also found in Cs distribution of each leaf position among the same rice cultivars. There has been no report clarifying the molecular mechanism of Cs translocation, nor those of other cations, in plants. Using the rice cultivars, Akihikari and Milyang23, to find the Cs translocation mechanism can contribute to developing crops that contain lower levels of Cs when cultivated in radiocesium contaminated land.

Keywords Breeding · Brown rice · Cesium · Grain · Fukushima Daiichi Nuclear Power Plant Accident · Rice · Translocation

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3.1 Introduction

In March 2011, a 9.1 magnitude earthquake occurred in Eastern Japan, triggering an extremely large tsunami. Consequently, the Tokyo Electric Power Company's Fukushima Daiichi Nuclear Power Plant (TEPCO-FDNPP) was unable to withstand the pressure exerted upon it by both forces resulting in a nuclear meltdown and radioactive contamination of the area surrounding the power plant. The radiocesium isotopes (^{137}Cs and ^{134}Cs) are of most concern for local agriculture because of their relatively long half-lives ($^{137}\text{Cs} = 30.2$ years; $^{134}\text{Cs} = 2.06$ years).

Because rice is the primary staple food in Japan, we have been particularly concerned over the rice crop in the fallout area. All rice bags produced in Fukushima have been inspected by screening equipment that was specifically designed for 30 kg rice bags (Nihei et al. 2015). Inspections have indicated that, after 2016, no single rice bag had radiocesium concentrations higher than the standard in Japan (100 Bq/kg; Table 3.1). We can confirm, finally, that this rice is safe to consume.

There are many reports supporting the mediation of Cs^+ transport via potassium ion (K^+) channels in root systems (Kim et al. 1998; Qi et al. 2008). In *Arabidopsis*, *AtHAK5* is the most well-known K^+ channel among numerous genes that transport Cs^+ (Qi et al. 2008; Nieves-Cordones et al. 2017; Ishikawa et al. 2017; Rai et al. 2017). Qi et al. (2008) reported that *AtHAK5* transports Cs^+ in plants under conditions of low K^+ availability. In rice plants, there have been reports that *OsHAK1*, expressed in roots under low potassium conditions, is involved in Cs^+ uptake from paddy soils (Nieves-Cordones et al. 2017; Ishikawa et al. 2017; Rai et al. 2017). We grew the *athak5* null mutant on Fukushima soil and determined that the ^{137}Cs in shoots was drastically decreased compared with that observed in wild-type shoots (Fig. 3.1).

When trying to clarify the mechanism of Cs accumulation in grain, Cs absorption by roots is not the only issue to be considered. The incident at the TEPCO-FDNPP occurred in March, which means that paddy soils were contaminated with radiocesium before any rice was planted in May. After planting the rice cultivar, the radiocesium in the paddy soil was absorbed by rice roots, and consequently, translocated to the grains. However, in March 2011, wheat was growing in the field as the nuclear crisis unfolded and leaves of the wheat were contaminated directly by radiocesium. The radiocesium concentrations in wheat grains grown in the same field correlated with wheat leaf mass at the time the fallout occurred, suggesting that ^{137}Cs translocation from leaf to grain was the main pathway for contamination of the wheat product at that time (Fig. 3.2). If a similar incident occurs during the rice growing season, radiocesium contamination directly to rice leaves would have a greater impact on rice grains via translocation; therefore, it is necessary to clarify the Cs translocation mechanism to breed low-Cs rice. However, in contrast to K^+ and Cs^+ absorption in roots, there is no molecular information regarding a transporter that mediates transport of K^+ or Cs^+ in above-ground biomass.

In the present study, we focused on Cs distribution in rice plants and tried to obtain a low-cesium phenotype by analyzing Cs translocation in different rice

Table 3.1 The inspection of all rice in all rice bags performed in Fukushima prefecture

Cultivation year	2012	2013	2014	2015	2016
Inspection period	08/25/2012~07/10/2015	08/22/2013~03/26/2015	08/21/2014~07/20/2016	08/20/2015~02/08/2017	08/24/2016~06/23/2017
Number of total rice bags	10,346,169	11,006,551	11,014,971	10,498,715	10,259,868
Number of rice bags containing 100 Bq/kg	71	28	2	0	0

Nihei et al., Sci. Rep. 2015, web site of Fukushima Association for securing safety of agricultural products

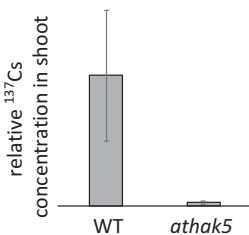


Fig. 3.1 Relative ¹³⁷Cs concentration in shoot. ¹³⁷Cs concentrations in shoots were drastically decreased in the *oshak5* null mutant when the plants were grown on the same Fukushima soil with low K⁺. The exchangeable K in the soil was 6.7 mg K/100 g soil. Data represents the mean ± standard deviation (Welch's t test: P = 0.0050)

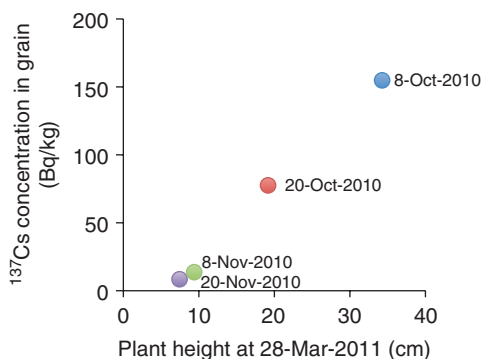


Fig. 3.2 ¹³⁷Cs concentrations in wheat grains produced in a field in Fukushima. The seeding dates were separated into four different 2010 plantings: October 8th, October 20th, November 8th, and November 20th. After the TEPCO-FDNPP incident occurred, the wheat plant heights were recorded on March 28th, 2011. The radiocesium concentrations of rice grains were measured after maturity at the end of June 2011. These data (in Japanese text) were provided by Arai Y., Nihei N., Takeuchi M. and Endo A., Fukushima Agricultural Technology Centre. The figure was modified from Tanoi (2013)

varieties. We selected three cultivars (Nipponbare, Akihikari, and Milyang23) based on the variation in radiocesium concentrations found in brown rice cultivated in Fukushima paddy fields in 2011 (Ono et al. 2014). Ono et al. studied 30 different cultivars, including 18 Japonica varieties, 2 Javanica varieties, and 10 Indica varieties. Results determined that the Nipponbare brown rice had low radiocesium concentrations, while that of Akihikari had high radiocesium concentrations among the Japonica varieties, and that the Milyang23, an Indica variety, had higher radiocesium concentrations than all other Japonica varieties (Ono et al. 2014). We grew the rice cultivars and separated the plantlets according to organ type (leaf, stem, peduncle, and ear) to measure the Cs concentrations within. Different distributions of Cs were found among the cultivars. These differences could provide a better understanding of both Cs⁺ and K⁺ translocation in rice plants.

3.2 Materials and Methods

3.2.1 ¹³⁷Cs Experiment to Grow the Three Rice Cultivars Hydroponically in a Growth Chamber

Seeds of three rice cultivars (*Oryza sativa* L. “Nipponbare,” “Akihikari,” and “Milyang23”) were soaked in water for 2–4 days and then transferred to a floating net in a 0.5 mM CaCl₂ solution. After 2 days, the seedlings were transferred to a 2-litre container with modified half-strength Kimura B nutrient solution (pH 5.6; Tanoi et al. 2011). Two-week-old rice seedlings were transferred to 300-ml pots with culture solutions containing ¹³⁷Cs (non-carrier-added ¹³⁷Cs; Eckert & Ziegler Isotope Products, Valencia, CA, USA). The plants were grown at 30 °C with a 12 h:12 h light: dark photoperiod. Culture solutions were changed twice per week.

Rice plants in the “heading” stage were collected and separated into organs (leaf, stem, peduncle, and ear). Each leaf number was set as an arbitrary ordinal number of leaves counted acropetally from an incomplete leaf on the main stem. When the grains had matured, we collected the ears and separated them into husk, brown rice, and rachis branch. The weight of each sample was measured after drying at 60 °C for 1 week. The radioactivity of each sample was measured using a well-type NaI(Tl) scintillation counter (ARC-300; Aloka Co., Ltd., Tokyo, Japan).

3.2.2 Paddy Field Experiment to Observe ¹³³Cs Distribution in Grains

Seeds of two rice cultivars (*Oryza sativa* L. “Akihikari” and “Milyang23”) were soaked in water for 2 days and then transferred to a seedbed in a greenhouse mid-April. Approximately 1 month later, the seedlings were transplanted to a paddy field in Tokyo (Institute for Sustainable Agro-ecosystem Services, Graduate School of Agricultural and Life Sciences, The University of Tokyo).

We analyzed ¹³³Cs instead of ¹³⁷Cs in the present field experiment. The matured rice grains were harvested in October. The grains were separated into husk and brown rice after being dried at 60 °C for more than 24 h. The samples were digested with 60% nitric acid for 3 h using the “Eco-Pre-Vessel system” (ACTAC; Tokyo, Japan). The digested solution was filtered using a 0.20 μm PTFE filter and diluted with deionized water to 5% nitric acid concentration. The concentrations of ¹³³Cs and ⁸⁵Rb were determined by inductively coupled plasma mass spectrometry using the ICP-MS 7500cx (Agilent Technologies) with ¹¹⁵In as an internal standard. The concentrations of K, Ca, Mg, and Na were determined from the digested solution using an inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 7300 DV, PerkinElmer). We analyzed nine plants for each cultivar.

3.2.3 ^{137}Cs Tracer Experiment Using Juvenile-Phase Rice

To observe the ^{137}Cs distribution in each leaf, we grew two cultivars Akihikari and Milyang23, in 250 ml of modified half-strength Kimura B solution (Tanoi et al. 2011) containing 1.8 kBq of ^{137}Cs at 30 °C for 16 days, until the seedlings had grown the 6th leaf after emergence. The solution was changed every other day. After the 16-day growth period, the shoots of the plants were separated into leaf sheaths and leaf blades for each leaf stage. After measuring the fresh weights, ^{137}Cs activity was measured in the samples using the well-type NaI(Tl) scintillation counter (ARC-300; Aloka Co., Ltd.). There were four replicates for each cultivar. In addition to the ^{137}Cs experiments, we prepared the same culture set without ^{137}Cs , digesting the leaf samples from the culture with 30% nitric acid using the DigiPREP system (GL Science; Tokyo, Japan). Concentrations of potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) were measured by ICP-OES (Optima 7300 DV; PerkinElmer).

To analyze ^{137}Cs uptake rate by roots, seedlings of Akihikari and Milyang23 cultivars that had grown the 6th leaf (about 16-day-old seedlings) were cultured in 200 ml of modified half-strength Kimura B solution containing 3.7 kBq of ^{137}Cs at 30 °C under lighted conditions for 30 min. After rinsing the root with tap water, the seedlings were washed with ice-cold half-strength Kimura B solution for 10 min. After cutting roots and shoots and measuring the fresh weight, the ^{137}Cs activities of the samples were measured using the well-type NaI(Tl) scintillation counter (ARC-300, Aloka Co., Ltd.). There were three replicates for each cultivar.

3.3 Results and Discussion

We grew our three chosen cultivars (Nipponbare, Akihikari, and Milyang23) in a culture solution containing ^{137}Cs inside a growth chamber. We then analyzed the ^{137}Cs distribution twice, at the heading stage and at the mature stage. When we measured the distribution at the heading stage, we found that the total amount of ^{137}Cs was lowest in Nipponbare and highest in Milyang23 (Fig. 3.3). We then separated the rice shoots into organs. When we analyzed the proportion of ^{137}Cs in shoots, ^{137}Cs concentrations in the ears of Milyang23 were twice as high compared to Nipponbare and Akihikari ears (Fig. 3.3). At that point we decided to focus on the ears, separating them into husks, brown rice, and rachis branches in the mature stage. We found that ^{137}Cs concentrations in husk and rachis branches were nearly the same between Akihikari and Milyang23, but the ^{137}Cs concentration in the brown rice from Akihikari was half that in Milyang23 (Fig. 3.4). The ^{137}Cs distribution suggests that ^{137}Cs translocation activity from leaves to brown rice occurs differently between Akihikari and Milyang23 varieties.

To confirm the different ^{137}Cs accumulation patterns between Akihikari and Milyang23 in field conditions, we grew these two cultivars in a paddy field in Tokyo

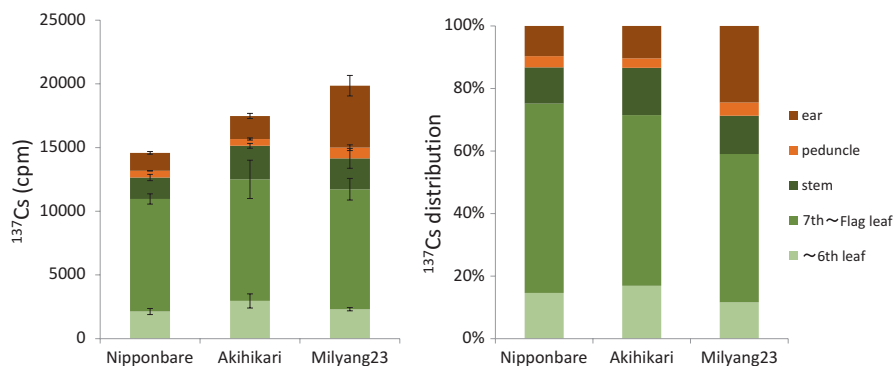
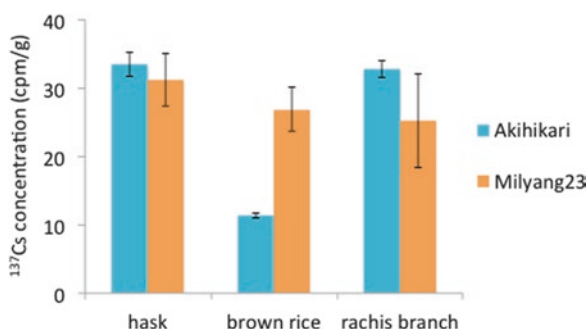


Fig. 3.3 ^{137}Cs amount in rice plants. Left: ^{137}Cs amount in the upper part of the plants. Right: ^{137}Cs distribution pattern in the upper part of the plants. Error bars: standard deviation

Fig. 3.4 ^{137}Cs concentration in ears of Akihikari and Milyang23. Error bars: standard deviation



(Fig. 3.5). In fact, the ^{137}Cs contamination in the paddy field was so low that the ^{137}Cs in grain was at an undetectable level, and we resorted to measuring ^{133}Cs instead, which confirmed our laboratory results, as described below.

The ^{133}Cs concentrations in brown rice from Akihikari were half those from Milyang23, while ^{133}Cs concentrations in the husks were comparable between the two cultivars (Fig. 3.6). Thus, Milyang23 showed preferential ^{133}Cs accumulation in brown rice over husk compared with Akihikari, which was consistent with our previous laboratory experiments using ^{137}Cs in a hydroponic culture.

There were no similar trends observed between Akihikari and Milyang23 for Rb (Fig. 3.6), K, Na, Ca or Mg concentrations (Table 3.2). The K concentration measured in husks from Milyang23 was double that of husks from Akihikari (Table 3.2). Mineral concentrations in the grain, showing no correlation between K and Cs, suggest that Cs concentrations in grain can be decreased without greatly deteriorating K concentrations simultaneously.

Translocation from old organs to new organs occurs in the juvenile phase. We analyzed 2-week-old plants of Akihikari and Milyang23 using ^{137}Cs . Before carrying



Fig. 3.5 Photos of the paddy field in Tokyo

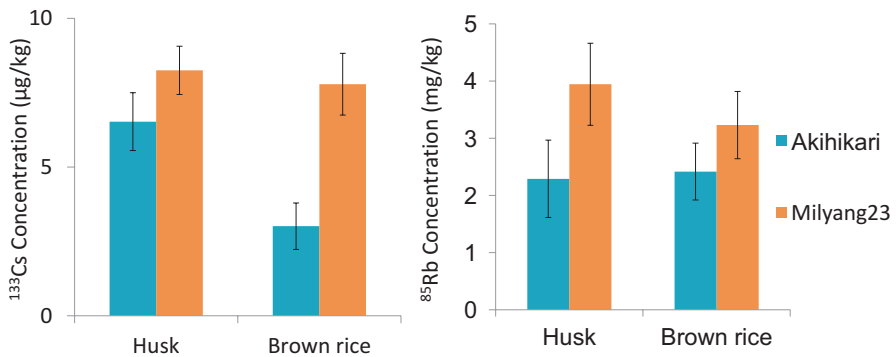


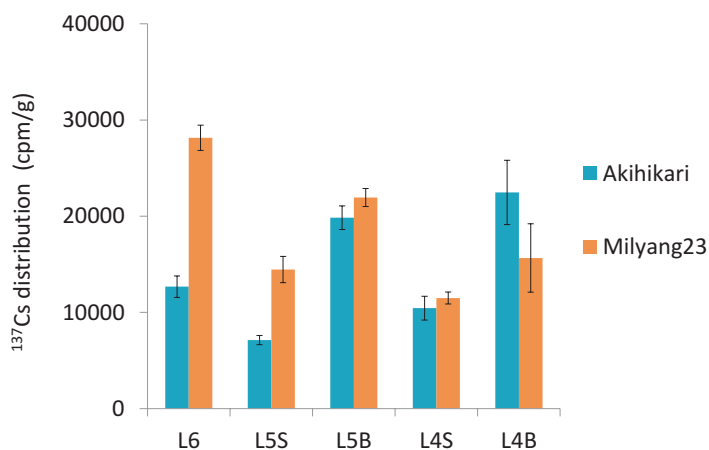
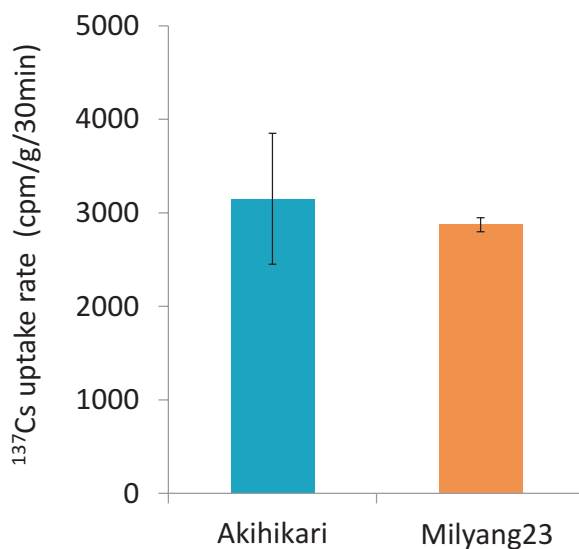
Fig. 3.6 ^{133}Cs and ^{85}Rb concentrations in husk and brown rice of Akihikari and Milyang23. Error bars: standard deviation

out the translocation experiments, we analyzed ^{137}Cs uptake rates in roots and determined that they did not differ between Akihikari and Milyang23 (Fig. 3.7).

Next, we analyzed the ^{137}Cs distribution in young rice plants at the leaf-6 stage. Results indicated that the ^{137}Cs concentration of L6, the newest leaf, was high in Milyang23 and low in Akihikari. On the other hand, the ^{137}Cs concentration of L4B, the oldest leaf blade among the leaves, was high in Akihikari and low in Milyang23 (Fig. 3.8). In general, minerals in leaves are transported via the xylem and phloem, and the phloem contribution is larger in newer leaves. In addition, minerals in old and mature leaves are translocated to new organs via the phloem. These results

Table 3.2 Mineral concentrations in brown rice and husk of Akihikari and Milyang23

	Brown rice		Husk	
	Milyang23	Akihikari	Milyang23	Akihikari
Ca (mg/kg)	0.064	0.095	0.58	0.46
Mg (mg/kg)	0.94	0.97	0.19	0.17
Na (mg/kg)	0.15	0.16	0.39	0.43
K (mg/kg)	1.0	1.1	2.2	1.1

Fig. 3.7 ^{137}Cs uptake rate of Akihikari and Milyang23. Error bars: standard deviation**Fig. 3.8** ^{137}Cs amount in leaves of Akihikari and Milyang23. L4, L5 and L6 means 4th leaf, 5th leaf and 6th leaf, respectively. B: blade, S: sheath. L6 is a blade on the 6th leaf whose sheath was too small to collect. Error bars: standard deviation

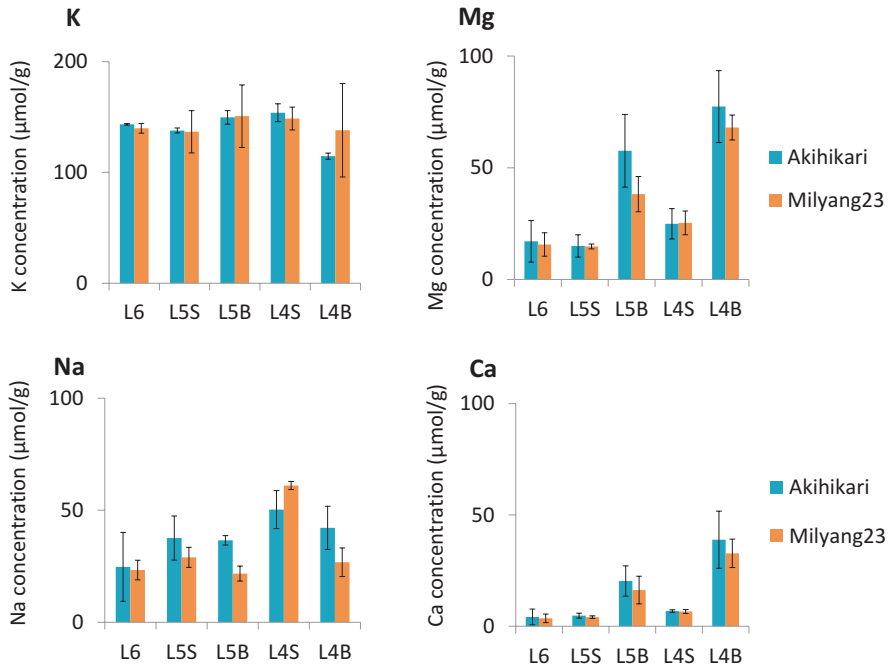


Fig. 3.9 Mineral concentrations in leaves of Akihikari and Milyang23. Error bars: standard deviation

indicate that Cs translocation from old mature leaves to new leaves is more vigorous in Milyang23 than Akihikari in the juvenile phase.

We also analyzed other minerals in the leaves, but there were no differences in mineral distribution between Akihikari and Milyang23 (Fig. 3.9).

To our knowledge, all the transporters reported to mediate Cs⁺ transport *in planta* were K⁺ channels (Qi et al. 2008; Ishikawa et al. 2017; Nieves-Cordones et al. 2017; Rai et al. 2017); therefore, the candidate transporters involved in Cs translocation should be K⁺ channels. Currently, however, there are no reports elaborating on the molecular mechanisms of K⁺ translocation in the upper part of a plant. Using the two cultivars, Akihikari and Milyang23, it may be possible to find the translocation system, not only of Cs⁺ but also of K⁺.

The low-Cs phenotype in the present study is not related to K concentrations. Clarifying the mechanism establishing this phenotype would contribute to breeding low-Cs crops without decreasing K concentrations and, consequently, without lessening the quantity and quality of the grains.

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