

Chapter 5

Time-Course Analysis of Radiocesium Uptake and Translocation in Rice by Radioisotope Imaging

Natsuko I. Kobayashi

Abstract The pattern of real-time ^{137}Cs uptake by plants was visualized using a macroscopic real-time radioisotope imaging system. We found that ^{137}Cs was easily taken up by rice plants only when it was dissolved in a liquid medium. In contrast, only a small amount of ^{137}Cs was taken up when the same liquid medium containing ^{137}Cs was added to the soil and supplied to the rice plants. This result demonstrates the intensive Cs adsorptive property of the soil. When rice was grown hydroponically in K-sufficient environment followed by K withdrawal for 2 days, ^{137}Cs was taken up easily compared with the rice without K deficiency. The application of K was shown to be an effective method for preventing radiocesium uptake by rice plants. However, K-deficiency was found to have little effect on the xylem loading process of Cs. Understanding the Cs translocation processes and evaluating the factors affecting each process based on experimental evidence could facilitate the development of an agricultural technique to reduce the radiocesium content in the edible parts of plants.

Keywords Imaging • K deficiency • Radiocesium • Rice • Tracer experiment • Xylem loading

Abbreviations

Cs Cesium
IP Imaging plate
K Potassium

N.I. Kobayashi (✉)
Graduate School of Agricultural and Life Sciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
e-mail: anikoba@mail.ecc.u-tokyo.ac.jp

| | |
|------------|---|
| Macro RRIS | Macroscopic real-time radioisotope imaging system |
| P | Phosphorus |
| PSL | Photo-stimulated luminescence |
| ROI | Region of interest |

5.1 Introduction

Limiting radiocesium uptake by plants from soil is important for reducing the ingestion of this pollutant. In addition to taking measures for preventing further entry of radiocesium into farmlands, inhibiting the soil-to-plant transfer of radiocesium by developing appropriate agricultural techniques may be effective. Cs belongs to the alkali metal group and has chemical and physical properties similar to other alkali metals. In plants, Cs is known to show similar behavior to K, which is an alkali metal and an essential macronutrient for organisms. Plants take up Cs through K^+ transporters and the K^+ channel pathway, and increase in the K concentration in a culture medium reduces the Cs uptake by plants due to the competition between K^+ and Cs^+ (Zhu and Smolders 2000). The exchangeable K^+ concentration in paddy soil showed a significant negative correlation with the radiocesium content in brown rice produced in the Fukushima region in 2011 (http://www.pref.fukushima.jp/keieishien/kenkyuukaihatu/gijyutsufukyuu/05gensiryoku/240112_tyukan.pdf). Thus, it was assumed that K fertilization to maintain the soil K^+ concentration at approximately 250 mg/kg, which is the recommended level suggested by the government, would be an effective technique for reducing the soil-to-plant transfer of radiocesium.

Preventing radiocesium accumulation, particularly in the edible parts of plant, may further reduce the risk of food contamination. Cs behaves similar to K in plants, as mentioned above. However, it is not known whether Cs behaves exactly the same as K in plants because it has not been sufficiently investigated and its behavior may differ depending on the species. A different Cs:K concentration ratio among tissues has been found in several grasses (Menzel and Heald 1955) and trees (Gommers et al. 2000; Robison et al. 2009), whereas the Cs:K concentration ratio among tissues was almost uniform in some tropical trees (Staunton et al. 2003). Furthermore, Robison et al. (2009) reported that the radiocesium distribution pattern within a coconut tree was altered by K fertilization. In *Arabidopsis*, mutants lacking individual K transporters had a specific Cs:K concentration ratio in their shoots (Hampton et al. 2005), indicating a relationship between specific properties of the K transport system and the Cs distribution pattern.

According to these studies, it may be necessary to understand the primary processes of Cs translocation separately within plants, such as the root uptake, xylem loading, and remobilization, before elucidating the Cs translocation mechanism and developing an agricultural technique for reducing the radiocesium content in edible parts. Therefore, we performed several types of ^{137}Cs tracer experiments in our laboratory.

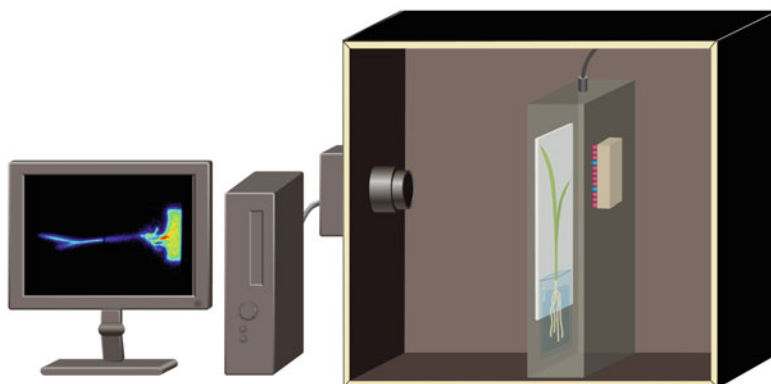


Fig. 5.1 Macro RRIS. The system consisted of a plant box with a fiber optic scintillator, a signal detection and multiplication component, and the monitor. The system had a viewing area of 10 cm×20 cm

5.2 Application of Macro RRIS for Quantification of ^{137}Cs Signals

To understand each translocation process separately, the real-time imaging analysis of ^{137}Cs migration through a whole plant could provide convincing information based on the non-destructive and continuous analysis of a single plant. Therefore, we evaluated the performance of a macroscopic real-time radioisotope imaging system (macro RRIS; Fig. 5.1; Nakanishi et al. 2009; Kanno et al. 2012) for ^{137}Cs determination. The results showed that macro RRIS, using an Al sheet to cover the scintillator surface as light shielding when detecting ^{137}Cs in illuminated conditions, could be used to image a spot containing 5 Bq ^{137}Cs in 1 μl during an integration period of 5 min (Fig. 5.2a). The detection performance of macro RRIS was lower than that of an imaging plate (IP) in terms of its sensitivity in the low radioactivity range (<10 Bq) and quantitative performance in the high radioactivity range (>400 Bq), but we demonstrated that macro RRIS could be applied efficiently for real-time imaging analysis using ^{137}Cs within the proper radioactivity range (Fig. 5.2b).

5.3 Uptake of ^{137}Cs by Rice Plants from Liquid or Soil Media

The strong adsorption of radiocesium by soil components, particularly clay, is known to occur immediately after radioactive fallout. This was clearly demonstrated by the imaging analysis (Fig. 5.3). When ^{137}Cs was added to the liquid medium, a large amount of ^{137}Cs , which was sufficient to produce a ^{137}Cs distribution image during an integration period of 10 min, was absorbed by the rice root and translocated to the shoot (Fig. 5.3a). In contrast, when the same amount of ^{137}Cs was added to the

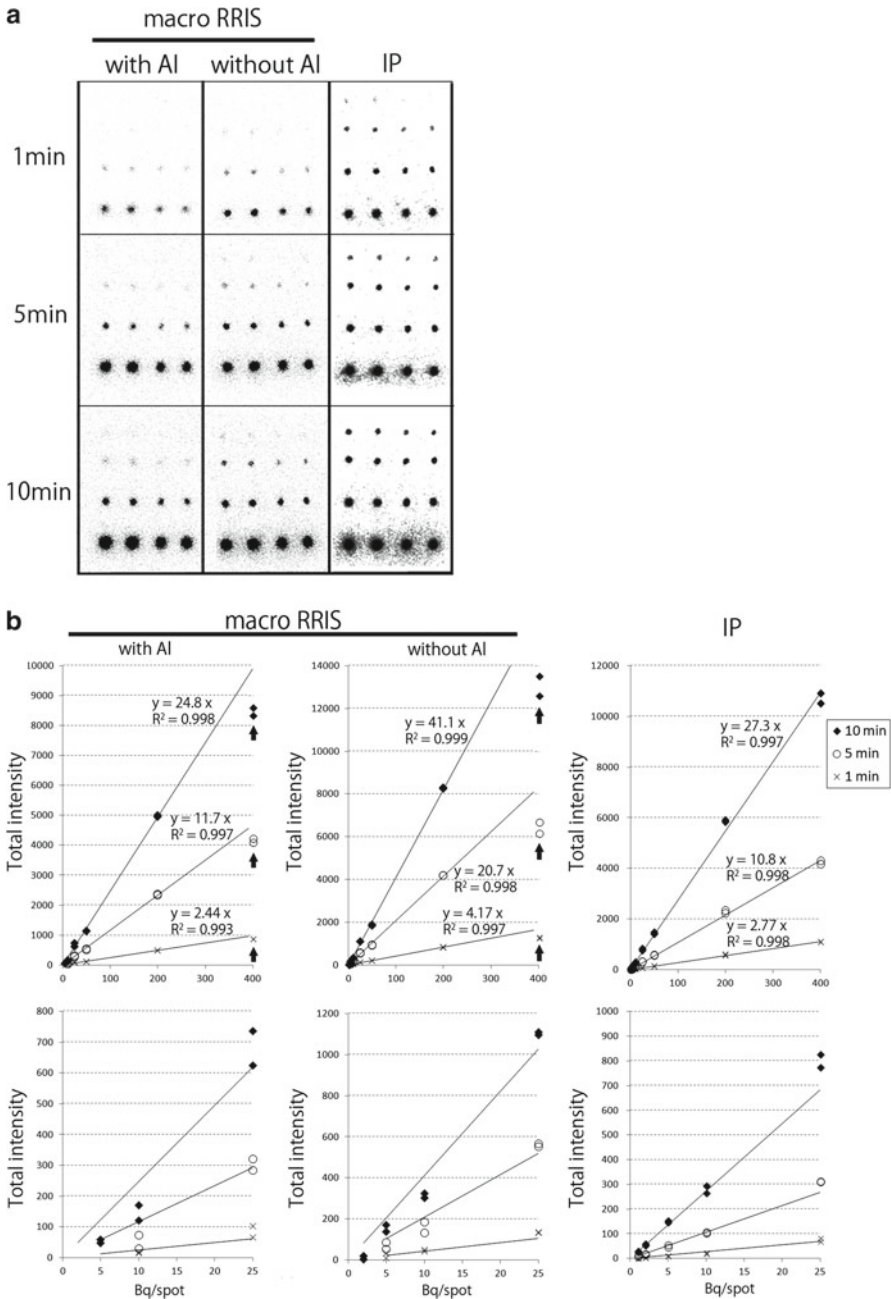


Fig. 5.2 Comparison of performance of macro RRIS and IP in the analysis of ^{137}Cs . **(a)** Images of a ^{137}Cs standard solution spotted on paper at concentrations ranging from 1 Bq (*top right*, two spots) to 400 Bq (*bottom left*, two spots), which were analyzed using macro RRIS and IP. Effects of the Al plate covering the scintillator surface were also examined. The standard solution was

soil medium, the rice plants absorbed only a little amount of ^{137}Cs even after 20 h, which was slightly detectable by IP (Fig. 5.3a, b). Immediately after the introduction of ^{137}Cs into the liquid medium, the ^{137}Cs signal in the liquid medium started to decrease and reached a plateau within 5 h, demonstrating that almost all ^{137}Cs added to the medium was absorbed by the rice (Fig. 5.3c). In the leaves, the ^{137}Cs signal increased almost linearly for 5 h, after which the rate of increase declined drastically, although plenty of ^{137}Cs was still present in the root (Fig. 5.3b). This characteristic ^{137}Cs uptake and translocation pattern was investigated further and has been presented in the following paragraph.

5.4 Distribution Pattern of Radiocesium in Soil

When ^{137}Cs was added to the soil, the ^{137}Cs signal was distributed uniformly throughout the soil (Fig. 5.4). However, radiocesium was found to be distributed non-uniformly in the surface soils collected from the Fukushima region, where some particles showed intense radioactivity (Fig. 5.4). The different radiocesium distribution patterns in the ^{137}Cs -added soil and the field soils contaminated by fallout suggested that the ^{137}Cs status of soil, particularly its chemical form, may be different, although the same radioactivity concentration was detected in both soils. This also implied that the results obtained in the soil-to-plant transfer analysis using soil with ^{137}Cs may not accurately reflect the natural situation in the field after contamination by fallout.

5.5 Observation of the Xylem Loading Activity Using Macro RRIS

As mentioned in Sect. 5.3, the rate of increase in the ^{137}Cs content declined significantly several hours after complete absorption of ^{137}Cs (Fig. 5.3c). There are two possible explanations for this observation. One is a decline in the physiological activity of the plant due to the specific experimental conditions, e.g., uncontrolled humidity, the possibility of severe mineral deficiency after the ^{137}Cs decline in the liquid medium within several hours (Fig. 5.3c), and the slight bending of the leaf blade within the plant box (Fig. 5.3a). If this was the case, only the initial increase in the curve could represent the physiological activity due to a combination of the



Fig. 5.2 (continued) detected using macro RRIS with an integration time of 1, 5, or 10 min, or using IP by maintaining contact with the spot for 1, 5, or 10 min. **(b)** Calibration curve for ^{137}Cs detected by macro RRIS with or without AI, and by IP. The signal intensity detected by macro RRIS and the PSL value recorded by IP were quantified based on the images in **(b)**. The approximate curves and their approximate linear equations are also presented. The area between 1 and 25 Bq is magnified and shown in the *bottom* row

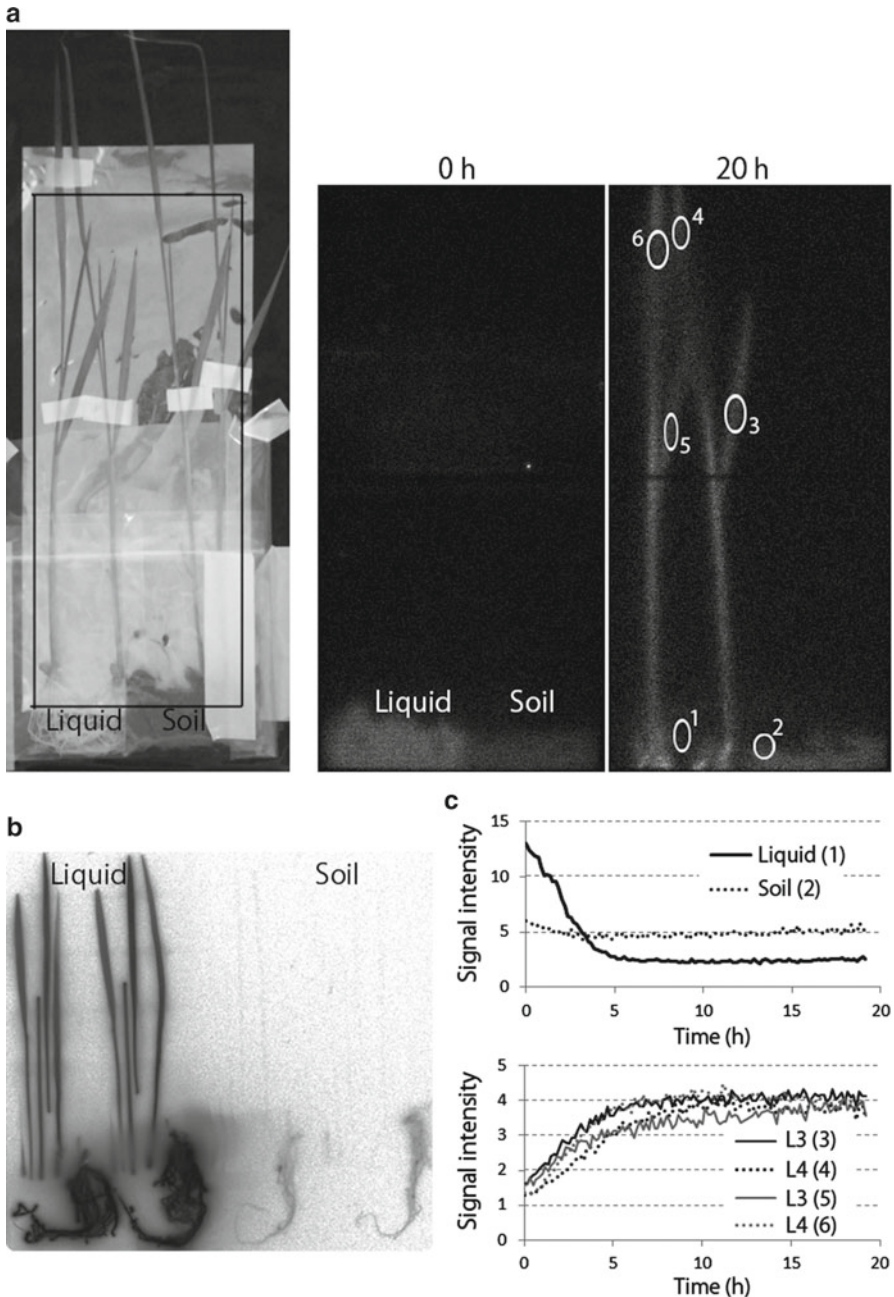
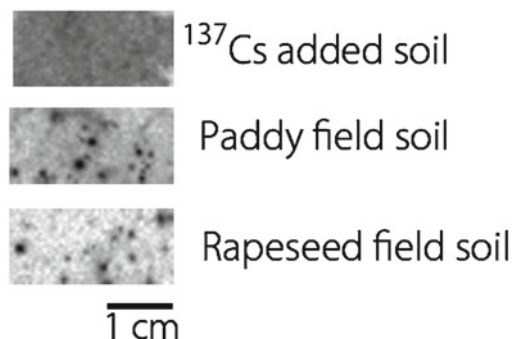


Fig. 5.3 ¹³⁷Cs uptake by the rice seedlings planted in liquid or soil. (a) Rice seedlings placed in the plant box and real-time images captured by the macro RRIS at the start of the experiment (0 h) and the end of the experiment (20 h). The integration time for each image was 10 min. The seedlings were grown in half-strength Kimura B liquid medium (K⁺=0.27 mM) until they developed three expanded leaves (L2, L3 and L4) under 16:8 h of *light:dark* conditions at 30 °C. Two seedlings

Fig. 5.4 The IP images of the soil with ^{137}Cs , and the surface soils collected from the paddy field and the rapeseed field in Fukushima district in June 2011. Bar = 1 cm



uptake and the xylem loading activity. Another explanation was that the ^{137}Cs increase in the shoot was composed of two distinct components. After the ^{137}Cs entered the root cytosol, some of the ^{137}Cs entered the cellular compartment for storage, whereas another part of the ^{137}Cs entered the xylem immediately and was translocated to the shoot through the xylem. If this was the case, the rapid translocation of ^{137}Cs to the shoot within 5 h (Fig. 5.3c) can be interpreted as the xylem loading activity, and the slowly increasing curve that appeared after 5 h (Fig. 5.3c) may be explained by the ^{137}Cs remobilization activity.

To distinguish these two possibilities, ^{137}Cs was resupplied to rice seedlings that had already been treated with ^{137}Cs for 19 h (Fig. 5.5). Then, ^{137}Cs content in the shoot increased again (Fig. 5.5). In contrast, the ^{137}Cs content did not change when the liquid medium was replaced with fresh medium that lacked ^{137}Cs , i.e., the renewing of experimental conditions did not restore the ^{137}Cs translocation from root-to-shoot (Fig. 5.5). Based on this result, the initially increasing curve was assumed to represent the xylem loading activity.

5.6 Effects of K Deficiency on the ^{137}Cs Uptake and Xylem Loading Activity

K-deficient plants usually accumulate greater amounts of Cs than K-sufficient plants (Zhu and Smolders 2000). A similar phenomenon was observed when K-sufficient and K-deficient rice seedlings were supplied with equal amounts of



Fig. 5.3 (continued) were planted in 3 ml of liquid medium or the soil medium (3 g of soil plus 3 ml of liquid medium) containing 50 kBq ^{137}Cs . The soil was collected from a paddy field in Fukushima district. The soil contained no radiocesium derived from fallout because the soil was collected from a deep part of the paddy field (5–10 cm from the surface). In the real-time image, six ROIs are shown. ROIs 1 and 2 indicate the liquid medium component. ROIs 3 and 5 are the L3 blades, whereas ROIs 4 and 6 are the L4 blades. **(b)** The IP image of rice seedlings after macro RRIS imaging was completed. The leaves were separated and cut into a root part with the bottom part of the shoot, L2, L3 sheath, L3 blade, L4 sheath, L4 blade, and L5 blade (L5 had not developed its sheath yet). The ^{137}Cs signal was hardly detected in L2 and L3 sheath. **(c)** Time course of ^{137}Cs signal accumulation in the six ROIs

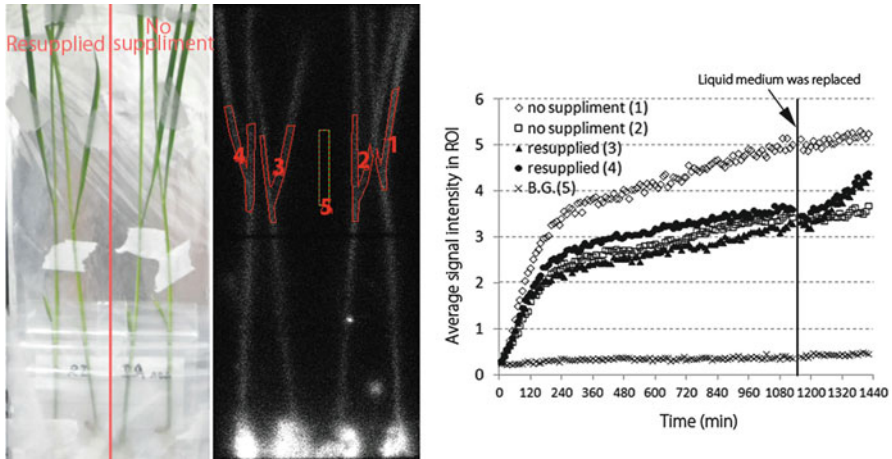


Fig. 5.5 Time course of ^{137}Cs signal accumulation in rice leaves after the liquid medium ($\text{K}^+=0.27\text{ mM}$) containing ^{137}Cs was replaced by liquid medium with or without ^{137}Cs . ^{137}Cs uptake images of four rice seedlings grown hydroponically for 2 weeks captured 19 h (1,140 min) after ^{137}Cs was introduced into the liquid medium at an intensity of 10 kBq/ml. Next, ^{137}Cs was resupplied for 4.5 h to two seedlings by replacing the liquid medium with 2 ml of fresh liquid solution containing 20 kBq ^{137}Cs . For the other two seedlings, the liquid medium was replaced by the fresh liquid medium without ^{137}Cs (no supplement). The integration time for each image was 10 min. ROIs 1 and 2 are the rice shoots without ^{137}Cs supplementation. ROIs 3 and 4 are the rice shoots that had ^{137}Cs resupplied at 1,140 min. ROI 5 represents the background signal intensity

^{137}Cs in a 0.27 mM K^+ condition (Fig. 5.6a). ^{137}Cs was rapidly accumulated in the K-deficient rice root (Fig. 5.6b), demonstrating that the ^{137}Cs uptake activity was increased significantly by a 2-day K-deficiency treatment. This increase in the ^{137}Cs uptake activity was quantified to determine the signal intensity in the liquid medium of K-deficient plants, where the ^{137}Cs signal decreased rapidly (Fig. 5.6c, d). However, the increase in the ^{137}Cs content of the leaves was not affected significantly by K-deficiency (Fig. 5.6d). This suggested that the ^{137}Cs xylem loading activity was not affected by the 2-day K-deficiency treatment. Alternatively, it is possible that K deficiency actually enhanced the xylem loading activity and that it also inhibited transpiration from the leaves, which could have negatively affected the root-to-shoot translocation of elements and the translocation rate to the shoots of K-deficient plants resulted in a similar manner to the K-sufficient plants. However, this possibility was rejected because the leaves of K-sufficient and K-deficient plants had the same transpiration rate (Table 5.1). In conclusion, 2 days of K deficiency enhanced the Cs uptake by the roots, whereas it had little effect on the Cs xylem loading process. Engels and Marschner (1992) suggested

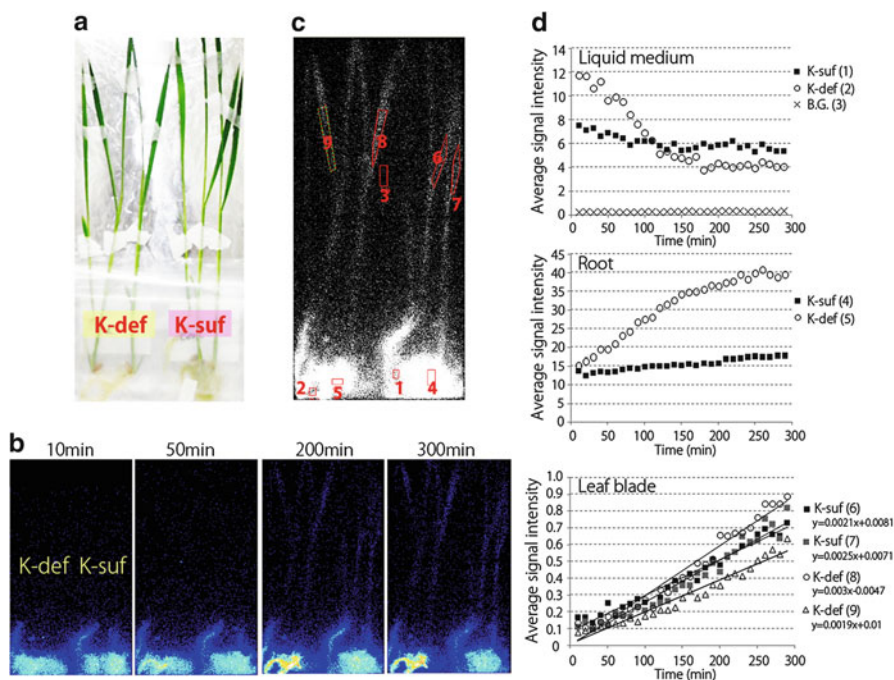


Fig. 5.6 Effects of K deficiency on the ^{137}Cs uptake and translocation pattern. (a) Rice samples placed in the plant box. The red flame indicates the viewing field of the macro RRIS. Three K-sufficient seedlings and 3 K-deficient seedlings were prepared, although we only analyzed two seedlings in each set. K-sufficient plants were grown in liquid medium containing 3.5 mM K^+ for 2 weeks. For the K-deficiency treatment, the rice seedlings were grown in the K-sufficient medium for 12 days and transplanted to the K-free liquid medium, before further culture for 2 days. The K-sufficient and K-deficient seedlings were treated with normal liquid medium with added ^{137}Cs (10 kBq/ml). (b) Part of the sequential images captured by macro RRIS at 5 h (300 min). The integration time for each image was 10 min. (c) Positions of nine ROIs. ROIs 1 and 2 are the liquid medium component. ROI 3 represents the background signal intensity. ROIs 4 and 5 are the roots. ROIs 6–9 are the L3 blades of each seedling. (d) Time course of the ^{137}Cs signal decrease in the liquid medium (top), and its accumulation in the root (middle) and the leaf blade (bottom). The approximate curves are shown for the ^{137}Cs signal in the leaf blade and the linear approximate equations are shown under each sample name. *K-def* K-deficient, *K-suf* K-sufficient

Table 5.1 Transpiration rates from leaf blades L3 and L4 of K-sufficient and K-deficient rice plants

| | L3 | | L4 | |
|---|-------|--------|--------|-------|
| | K-suf | K-def | K-suf | K-def |
| Transpiration rate (mmol $\text{H}_2\text{O}/\text{m}^2/\text{s}$) | 1.17 | 1.02 | 1.14 | 1.45 |
| SD | 0.189 | 0.0687 | 0.0833 | 0.201 |

No significant difference was observed between the samples

Sample size=3; SD is the standard deviation of the three samples; *K-suf* K-sufficient, *K-def* K-deficient

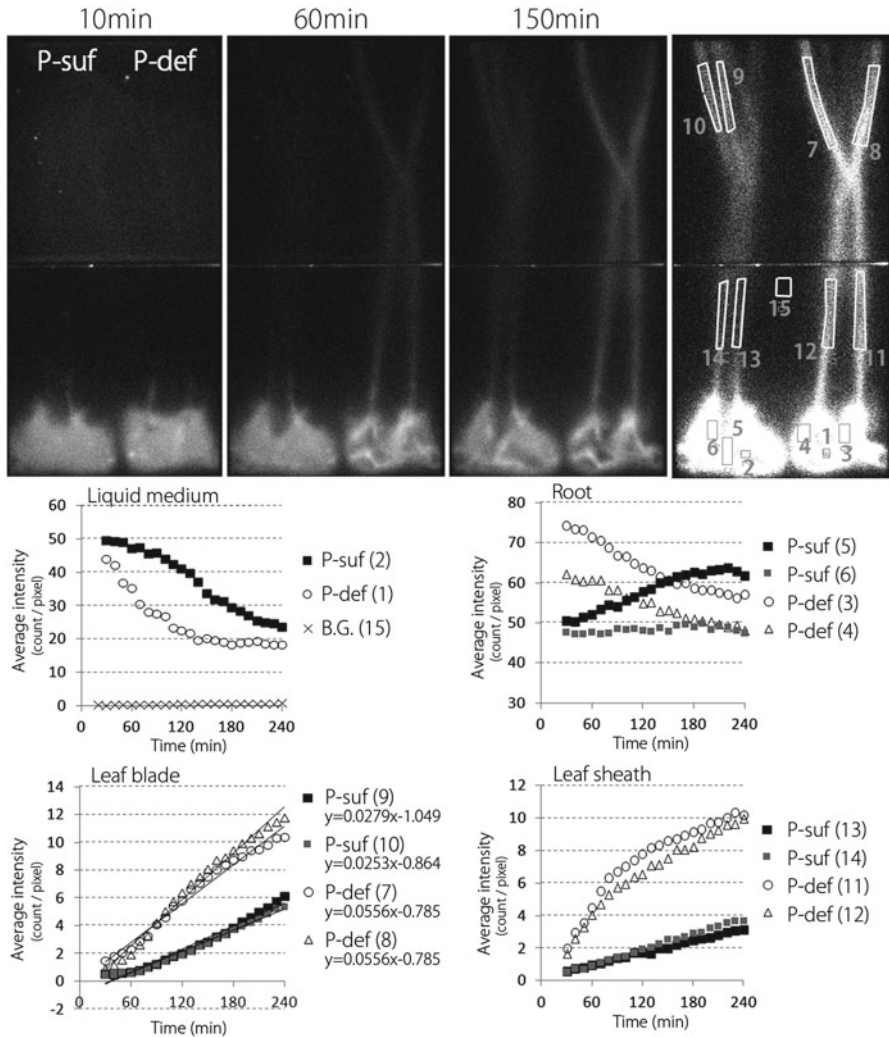


Fig. 5.7 Effects of P deficiency on ^{32}P uptake and translocation. For the P-sufficient and P-deficient samples, two seedlings were grown in normal half-strength Kimura B liquid medium (phosphate = $92\ \mu\text{M}$) for 2 weeks and two seedlings were grown normally for 6 days followed by cultivation in P-free liquid medium for 6 days, respectively. The P-sufficient and P-deficient seedlings were then placed into normal liquid medium containing ^{32}P ($2\ \text{kBq/ml}$). After recording the ^{32}P images for 4 h, 14 ROIs were selected, i.e., the liquid medium component (ROIs 1 and 2), the roots (ROIs 3–6), the leaf blades (ROIs 7–10), and sheaths (ROIs 11–14), as indicated, to determine any time-dependent changes in the signal intensity. ROI 15 represents the background signal intensity. The approximate curves and their linear approximate equations are added in the corresponding figure to demonstrate the ^{32}P translocation kinetics in the leaf blade. *P-suf* P-sufficient, *P-def* P-deficient

that the K-xylem loading process and root uptake process were regulated separately in maize. Assuming that Cs migration was basically regulated by the K control system, the results presented in this study are consistent with those reported by Engels and Marschner (1992).

5.7 Enhanced P Xylem Loading Activity in P-Deficient Rice Plants

With respect to the xylem loading activity, it is known that the root-to-shoot transport of P is induced by P deficiency through the upregulation of P transporter gene expression (Ai et al. 2009). To confirm this property of P using macro RRIS and to confirm our experimental characterization of the xylem loading activity, we analyzed the ^{32}P uptake and translocation pattern using macro RRIS. After comparing the declining curve of the ^{32}P signal in the liquid medium from P-sufficient rice plants with that of P-deficient plants, we confirmed the positive effect of P-deficiency on the phosphate uptake activity (Fig. 5.7). After being absorbed by the roots, phosphate was shown to be transported to the shoots immediately and it never accumulated in the roots of P-deficient rice (Fig. 5.7). There was an evident increase in the P content of the leaves of P-deficient rice. These observations confirmed the stimulatory effect of 6 days of P deficiency on the P-xylem loading activity and uptake activity.

5.8 Conclusion and Future Perspectives

Using macro RRIS, we analyzed Cs uptake and xylem loading processes separately. In contrast to the positive effect of P deficiency on the uptake and xylem loading of P, K-deficiency had an initially positive effect on Cs uptake but not on Cs-xylem loading process. Other than these processes, the remobilization of Cs in the leaves and roots, the xylem-to-phloem transfer activity, and Cs exclusion from the roots are suggested as potential factors that affected the radiocesium distribution patterns in plants. Given that the Cs distribution in the edible parts is known to vary depending on the plant species, understanding these Cs translocation processes and evaluating the environmental factors that affect the Cs distribution pattern in rice, a model plant, could help to develop crop-specific agricultural techniques for reducing radiocesium accumulation in the edible plant parts. Further tracer experiments are required to evaluate each Cs translocation process and are currently underway.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Ai P, Sun S, Zhao J, Fan X, Xin W, Guo Q, Yu L, Shen Q, Wu P, Miller AJ, Xu G (2009) Two rice phosphate transporters, OsPht1;2 and OsPht1;6, have different functions and kinetic properties in uptake and translocation. *Plant J* 57:798–809
- Engels C, Marschner H (1992) Adaptation of potassium translocation into the shoot of maize (*Zea mays*) to shoot demand: evidence for xylem loading as a regulating step. *Physiol Plant* 86:263–268
- Gommers A, Thiry Y, van den Hove H, Vandecasteele CM, Smolders E, Merckx R (2000) Radiocaesium uptake by 1-year-old willows planted as short rotation crop. *J Environ Qual* 29:1384–1390
- Hampton CR, Broadley MR, White PJ (2005) Short review: the mechanisms of radiocaesium uptake by *Arabidopsis* roots. *Nukleonika* 50:S3–S8
- Kanno S, Yamawaki M, Ishibashi H, Kobayashi NI, Hirose A, Tanoi K, Nussaume L, Nakanishi TM (2012) Development of real-time radioisotope imaging system for plant nutrient uptake studies. *Philos Trans R Soc B* 367:1501–1508
- Menzel RG, Heald WR (1955) Distribution of potassium, rubidium, caesium and strontium within plants grown in nutrient solutions. *Soil Sci* 78:287–293
- Nakanishi TM, Yamawaki M, Kanno S, Nihei N, Masuda S, Tanoi K (2009) Real-time imaging of ion uptake from root to above-ground part of the plant using conventional beta-ray emitters. *J Radioanal Nucl Chem* 282:265–269
- Robison WL, Brown PH, Stone EL, Hamilton TF, Conrado CL, Kehl S (2009) Distribution and ratios of ^{137}Cs and K in control and K-treated coconut trees at Bikini Island where nuclear test fallout occurred: effects and implications. *J Environ Radioact* 100:76–83
- Staunton S, Hinsinger P, Guivarch A, Brechignac F (2003) Root uptake and translocation of radiocaesium from agricultural soils by various plant species. *Plant Soil* 254:443–455
- Zhu Y-G, Smolders E (2000) Plant uptake of radiocaesium: a review of mechanisms, regulation and application. *J Exp Bot* 51:1635–1645