Chapter 19

Visualization of Ion Transport in Plants

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Abstract We have developed a real-time radioisotope imaging system (RRIS) to visualize ion transport in plants, and to measure radioactivity in living plants. To know the mechanisms of ion transport in plants, the use of living plants allows us to visualize ion movement in real time. In addition, the RRIS can analyze how a change to the plant environment affects ion transport. In this chapter, we will introduce some of the applications of the RRIS. We analyzed the effect of light on cesium, potassium, magnesium, phosphate, and calcium transport in plants using the RRIS. The results show that magnesium, potassium, and calcium transport in plants were not influenced by light. On the other hand, the amount of cesium and phosphate absorption in roots decreased after light-off. Moreover, the amount of phosphate transport from root to shoot also decreased after light-off.

Keywords Live imaging · Radiocesium · Rice · Xylem flow · Real-time radioisotope imaging

Abbreviations

Ca	Calcium
Cs	Cesium
K	Potassium
Mg	Magnesium
P	Phosphate
RRIS	real-time radioisotope imaging system

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19.1 Introduction

After the accident of Fukushima Daiichi nuclear power plant, it is necessary to reduce radiocesium (^{137}Cs) in crops. To solve this problem, it is important to know how much ^{137}Cs is accumulated in each tissue and to understand in detail the mechanism of ^{137}Cs transport in plants. There are two pathways responsible for elemental transport in plants, namely, the symplastic and apoplastic pathways. The symplastic pathway transports water and ions via the plasmodesma, whereas transport by the apoplastic pathway is via cell walls. In addition, transporters within membranes also affect elemental transport. Elements within the vascular bundle are distributed between tissues through vessels and sieve tubes. Water and nutrients are mainly transported via vessels and the driving force is root pressure and transpiration. Water, nutrients, and photosynthetic products are transported via sieve tubes and the driving force depends on the concentration gradient of sucrose. The environmental changes, such as weather, affect ion transport because the rate of transpiration and photosynthesis is influenced by light. Various techniques are available to researchers to analyze transport and distribution of ^{137}Cs in plants. In particular, we often use imaging techniques. One such imaging technique is the real-time radioisotope imaging system (RRIS) which was developed in our laboratory (Nakanishi et al. 2009). The RRIS can visualize transport and distribution of various radioisotopes including ^{137}Cs in living plants. In addition, we can analyze the amount of cesium using the images obtained by the RRIS. To analyze ion transport in plants using the RRIS with living plants is a big advantage because ions in plants are continuously moving. Some of the findings related to Cs transport include the following: Kobayashi visualized ^{137}Cs in rice plants grown in water culture and soil, and found minimal ^{137}Cs translocation to rice grain when grown in soil because of ^{137}Cs adsorption to soil particles (Kobayashi 2013). In addition, Sugita et al. reported that the transport manner of ^{137}Cs and ^{42}K from roots to above-ground parts of *Arabidopsis* is similar for ^{137}Cs and ^{42}K (Sugita et al. 2016). Kobayashi et al. demonstrated that ^{137}Cs was retained in the root tissues with high efficiency, while ^{42}K was easily exchanged and transported towards the shoots (Kobayashi et al. 2016). Moreover, RRIS can analyze how changes in the growth environment of living plants affect ion transport, such as temperature or nourishment. In this chapter, we will report the characteristics of the Cs transport compared to other elements in roots of rice plants when environmental conditions change from light to darkness.

19.2 Imaging System and Imaging Methods

19.2.1 Obtaining Images

The characteristics of the Real-time radioisotope imaging system (RRIS) are as follows:

Fig. 19.1 Overview of the mechanism of real-time radioisotope imaging system

The radiation from nuclide is converted to visible light by CsI (Tl) scintillator, and the visible light is then captured by CCD camera

This figure is quoted from Sugita et al. (2017)

- It is possible to visualize and quantify ion movements in living plants using radioisotopes;
- It is possible to detect many types of radiations such as beta-, X-, gamma-rays, and positron (Sugita et al. 2014)

There are two steps to visualize radiation. (1) Radiation is converted to visible light by CsI (Tl) scintillator, and (2) the visible light is captured by CCD camera (Fig. 19.1). RRIS can use living plants for experimentation, so we can get sequential images. The light converted from radiation is very weak, therefore in order to take images, it is necessary to use a dark conditions box.

19.2.2 Two Types of View Areas

The RRIS has two types of view areas. One is 10×20 cm in size (w $\times$ h) and known as the macro-RRIS. The other, the micro-RRIS, has the ability to take images within the μm range using a microscope. The view area is approximately $600 \times 600 \mu\text{m}$ (optical lens: x20 magnification) to 5.2×5.2 mm (x2.5 magnification) in size (W $\times$ L).

19.2.2.1 macro-RRIS

The macro-RRIS mainly visualizes ion movements in whole plants. Test plants are placed on the CsI (TI) scintillators (Fig. 19.2a). The dark box's size is approximately $120 \times 150 \times 80$ cm (W $\times$ L $\times$ D). We have taken images of rice, Arabidopsis, soy beans, poplar, for example.

19.2.2.2 micro-RRIS

The mechanism of how to take images by micro-RRIS is identical to how images were taken by macro-RRIS. The CsI (TI) scintillator (1.5×1.5 cm) is placed on the plant, and the light converted from radiation by the scintillator is captured by CCD camera through a microscope (Fig. 19.2b).

19.2.3 Radioisotopes

Commercially available radioisotopes are predominantly used for visualizing elements within plants. On the other hand, radioisotopes with short half-lives (e.g., ^{42}K and ^{28}Mg) have to be produced by our laboratory as they are not available commercially. For example, ^{42}K has a half-life of 12 h and ^{28}Mg has a half-life of 20 h.

19.2.3.1 ^{42}K

^{42}K is produced by ^{42}Ar - ^{42}K generators (Aramaki et al. 2015). The half-life of ^{42}Ar and ^{42}K is about 33 y and 12 h, respectively. The method of producing ^{42}K is described as follows (Fig. 19.3a): (1) a steel electrode is inserted into a generator

Fig. 19.2 Schematic drawing of the two types of real-time radioisotope imaging system (RRIS)

(a) Macro-RRIS; the view area is approximately 10×20 cm

(b) Micro-RRIS; the view area is approximately 600×600 μm (x20 magnification)

Fig. 19.3 Schematic drawing of the ^{42}Ar - ^{42}K generator
 (a) ^{42}K is attracted to the cathode when voltage is applied
 (b) ^{42}K is extracted from the cathode using water

with a cock which has ^{42}Ar . (2) ^{42}K is produced from ^{42}Ar when the steel electrode has a minus charge and the generator is positively charged. (3) When 60 V is applied to the electrode and generator, ^{42}K is attracted to the steel electrode. (4) the steel electrode is removed from the generator and ^{42}K is collected inside a pipette with water (Fig. 19.3b).

19.2.3.2 ^{28}Mg

^{28}Mg was produced using a cyclotron by ^{27}Al (α , 3p) ^{28}Mg reaction (Tanoi et al. 2013).

19.2.4 Applying Radioisotopes to Plants

Radioisotopes can be applied to plants either in gas or liquid form. Gas radioisotopes such as ^{14}C -labeled CO_2 (Sugita et al. 2013) can be applied to the whole plant or to only specific tissues (e.g., leaves, shoots, stems). Liquid radioisotopes such as ^{137}Cs and ^{42}K are applied to roots when test plants are grown in culture solutions, gels or soils. In addition, it is possible to visualize ion movement from a leaf to other tissues (Sugita et al. 2016).

19.2.5 Applying Light to Plants

The CCD camera in the RRIS has a high sensitivity, but its detector can become damaged if exposed to strong light. In addition, visible light converted from radiation is very weak, and therefore it is necessary to have complete dark conditions while taking images. Because plants need light for growth, an intermittent lighting system was developed (Hirose et al. 2013). This lighting system ensures dark conditions when images are being taken and light conditions for the remaining time. For example, one dark/light cycle is 3 min/7 min and the RRIS gets an image for 3 min in the dark condition. Moreover, the cycle can be set for 24 h, allowing the RRIS to visualize ion movements for 24 h. The micro-system has the same type of system.

19.3 Impact of Light for Ion Transport in Plants

19.3.1 Experimental

19.3.1.1 Test Plants

Rice seeds (*Oryza sativa*, L. 'Nipponbare') were germinated in 0.5 mM CaCl_2 solution under dark conditions for 2 days. The plants were transplanted into half-strength Kimura-B nutrient solution (270 mM K^+) for 6 days. Next, four plants were placed in a root chamber with half-strength Kimura-B nutrient solution. For ^{137}Cs imaging, 0.1 μM CsCl_2 was added to the Kimura-B nutrient solution. The root chamber was made of a polyethylene bag and polyurethane sheet (Fig. 19.4a). The plant roots were fixed inside the bag using the polyurethane sheet, and the bag was fixed onto the scintillator (Fig. 19.4b).

Fig. 19.4 The mounting arrangement of test plants in the real-time radioisotope imaging system (a) Four plants were placed in a root chamber. The roots were fixed using the polyurethane sheet (b) The root chamber was pressed onto the scintillator using acrylic resin plates
This figure is quoted from Sugita et al. (2017)

Table 19.1 General overview of the radionuclides used in this study
The value of each radiation is the maximum energy (keV)

Nuclide	Mode of decay	Half-life	β -ray	γ -ray
^{28}Mg	β^-	20.9 h	860	1589
^{28}Al	β^-	2.24 m	2863	1779
^{32}P	β^-	14.3 d	1711	—
^{42}K	β^-	12.4 h	3525	1525
^{45}Ca	β^-	163 d	257	—
^{137}Cs	β^-	30.2 y	1176	662
$^{137\text{m}}\text{Ba}$	IT	2.55 m	—	662

The ^{28}Al is daughter nuclide of ^{28}Mg

The $^{137\text{m}}\text{Ba}$ is daughter nuclide of ^{137}Cs

19.3.1.2 Visualization of Element Movement Using RRIS

The following radioisotopes were applied to the roots: ^{28}Mg : 17 kBq mL $^{-1}$; ^{32}P : 30 kBq mL $^{-1}$; ^{42}K : 7 kBq mL $^{-1}$; ^{45}Ca : 830 kBq mL $^{-1}$; and ^{137}Cs : 25 kBq mL $^{-1}$. Their characteristics are shown in Table 19.1. The total length of time for taking images was 10 h. During the first 5 h, the dark/light cycle of 3 min was set up using LED

lights ($100 \mu\text{mol s}^{-1} \text{ m}^{-2}$), and then the RRIS took images using 3 min of exposure time for each 3 min dark condition. During the next 5 h, the RRIS took images using 3 min of exposure time for each 10 min in the dark condition.

19.3.1.3 Transpiration Rate

Transpiration rate was calculated as the decreased level of the culture water under light for 10 h or under light condition for 5 h followed by 5 h of dark condition in the dark box of the RRIS.

19.3.2 Results and Discussion

RRIS was used to visualize the response of ion movement between light and dark conditions. At first, each radioisotope (^{137}Cs , ^{42}K , ^{28}Mg , ^{32}P , and ^{45}Ca) was supplied to roots and visualized for 5 h under light (0–5 h) and dark conditions (5–10 h) (Fig. 19.5a–19.5d). To analyze the changes during uptake in roots and translocation from root to shoot, the radioisotope activities were measured in the region of interest (ROI) of the shoot and root based on the RRIS images (Fig. 19.6a); the time course of signal intensity within the ROI was then obtained. The signal intensity value was shown relative to the 5 h after the radioisotope absorption (Fig. 19.6b–e). In addition, the slopes of the calibration curves were calculated for 3–5 h and 5–7 h to compare the radioisotope accumulation velocities (Fig. 19.6f). The results show that the ^{137}Cs activity in roots only increased slightly after turning the light off (Fig. 19.6b) and the slope of the calibration curve showed a statistically significant decrease (Fig. 19.6f). On the other hand, the activities in the shoots of ^{137}Cs were not influenced by light and continuously increased after turning the light off. These results suggest that Cs uptake from the root is largely influenced by light and Cs transport from root to shoot is not influenced by light. In regards to K, there was no influence of light on either shoot or root (Fig. 19.6b). These results suggest that in Cs absorption from roots, it is possible that transporters which are different to K transporter greatly contributes to Cs transport and the activity of those transporters are affected by light. Both ^{45}Ca and ^{28}Mg accumulation in shoot and root were not influenced by the change from light to dark conditions (Fig. 19.6c, e, f). For ^{32}P , the reaction from light to dark condition was prompt, with ^{32}P accumulation rate in both shoot and root decreasing (Fig. 19.6d). Considering the response time of transpiration from light to dark condition is several minutes (Ishikawa et al. 2011), it was assumed that transpiration greatly influenced ^{32}P transport. After 5 h from light to dark condition, transpiration decreased 57% in comparison with continuous light (Fig. 19.7). This result suggests that P was absorbed and transported with water, while Mg, Ca, and K were not affected by the flow of water. On the other hand, the

Fig. 19.5 Serial images under light/dark conditions in rice, taken by RRIS
After the radioisotope was added, the serial images were taken under light conditions for the first 5 h and dark conditions for the following 5 h
(a) ^{42}K - ^{137}Cs , (b) ^{28}Mg , (c) ^{32}P , and (d) ^{45}Ca
This figure is quoted from Sugita et al. (2017)

Fig. 19.6 The influence of ion movement under light/dark conditions (a) Photograph of test plants. The blue, red, and green lines indicate the region of interest (ROI) of shoot, root, and background, respectively. Time-course analysis of the radioactivity of (b) ^{42}K - ^{137}Cs , (c) ^{28}Mg , (d) ^{32}P , and (e) ^{45}Ca . The relative signal intensity was normalized at 5 h. (f) The slope of the calibration curve between 3–5 h (light) and 5–7 h (dark) based on Fig. b–e. Data represent means $\pm$ standard deviation ($n = 4$ plants). This figure is quoted from Sugita et al. (2017)

Fig. 19.7 Transpiration rate was calculated under the light condition for 10 h (circles), or under light condition for 5 h followed by 5 h of dark condition (crosses) at 3, 5, 7 and 10 h. Data represent means $\pm$ standard deviation ($n = 4$ plants). This figure is quoted from Sugita et al. (2017)

signal level of ^{45}Ca in the root only increased slightly after 3 h after its addition (Fig. 19.6e). This result shows that the replacement of Ca^{2+} with ^{45}Ca in roots is quick. In addition, ^{45}Ca accumulation in shoot continuously increased, suggesting that surplus ^{45}Ca is transported to the shoot.

As shown in the examples above, RRIS is a powerful tool to analyze responses in plants to their environment. To decrease ^{137}Cs accumulation in plants, we will continue to research the uptake and translocation of ^{137}Cs in plants using RRIS.

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