Chapter 4

Real-Time Element Movement in a Plant

Keywords Real-time RI imaging · Real-time RI imaging system · Microscopic RI imaging system · Rice · Soybean · Arabidopsis · ^{14}C · ^{22}Na · ^{28}Mg · ^{32}P · ^{33}P · ^{35}S · ^{42}K · ^{45}Ca · ^{54}Mn · ^{65}Zn · ^{109}Cd · ^{137}Cs · Image analysis · Root absorption image · Micro-movement · Imaging system development

4.1 Conventional Radioisotope (RI) Imaging

Radioisotope (RI) imaging has been developed as radiography using X-ray film. RI was supplied to the plant, and the plant was placed on an X-ray film for exposure to acquire the radiation image on the film. With further development of the technology for this kind of radiography, an IP (imaging plate) replaced the X-ray film, providing much higher sensitivity. Since the image by an IP showed a linear relationship between the whiteness in the image and the radiation intensity, it was easy to quantify the amount of the radionuclide from the image that appeared on the IP. With the development of gene technology, where ^{32}P was employed to label DNA, IP was widely used to detect the RI band of DNA, especially in electrophoresis.

Figure 4.1 shows how to obtain an RI image of the plant sample. After treatment with RI, the plant was placed in a cassette containing an X-ray film or an IP, and the film or IP was exposed to the radiation from the sample for a time. After exposure, the X-ray film was developed, and the image was acquired by a scanner. In the case of an IP, the image produced in the IP was acquired by an image scanner and stored in a computer. After scanning, the image in the IP could be erased, and the IP could be used repeatedly for other samples.

Figure 4.2 shows an example of an RI radiograph of a soybean plant 2, 4, and 6 days after pulse (2 h) treatment of the root with 0.325 kBq/mL of ^{109}Cd solution. Figure 4.2a is the image of the whole plant showing the distribution of ^{109}Cd . Figure 4.2b shows the dissection images of the plant taken by an IP, suggesting

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Fig. 4.1 RI (radioisotope) imaging system using an IP. A plant sample containing RI is placed on an IP shown as a white board, and the IP was exposed to radiation from the sample for a time period in a cassette. Then, the radiation image is scanned by a scanner, and the RI amount is analyzed by a computer

Fig. 4.2 An example of a ^{109}Cd image in a soybean plant. ^{109}Cd distribution in the whole plant (a) and the dissection (b) when ^{109}Cd was supplied under different pH conditions. (a): pseudocolor was added according to the intensity of the radiation; (b): darker colors indicate higher radioactivity

that Cd translocation was performed mainly via the vascular bundle. The resolution of the image obtained using an X-ray film was approximately $20\text{ }\mu\text{m}$, and that obtained using an IP was approximately $50\text{--}100\text{ }\mu\text{m}$.

These are the widely applied methods of RI radiography. To maximize the resolution and contrast of the image, the sample should be kept as close as possible to the film or the IP during the exposure. A cassette is usually used for exposure of

Fig. 4.3 Trunk cross section image of ^{137}Cs [1]. One year after the Fukushima nuclear accident, a cedar (*Cryptomeria japonica*) tree grown in the mountain in Fukushima, where the environmental radioactivity was approximately $0.2 \mu\text{Sv/h}$, was downed, and wood disks (5-mm thickness) were removed from the same log. The ^{137}Cs distribution image in the disk was acquired using an IP (BAS-IP MS, GE Healthcare Japan) after 1–5 months of exposure time. The image in the IP was scanned by a fluorescent image analyzer (FLA-9000, Fujifilm)

the film or IP. However, if a cassette is used, the same plant cannot be used for further experiments after being kept in the cassette, since the plant was pressed tightly by the cassette lid for a while.

As another example, a radiograph of a wood disk is presented, which was taken after the Fukushima nuclear accident (Fig. 4.3). The cedar tree was cut down; wood disks, 1 cm in thickness, were removed from different heights of the tree; and the radiation image of ^{137}Cs was taken by an IP. The radiographs showed that fallout ^{137}Cs accumulated at the trunk surface and heartwood, especially at the higher positions in the tree.

These are static images of harvested plant samples, taken by an IP. However, to obtain images of living plants, several devices are needed. For example, to determine the RI distribution in roots, the following device was prepared. A container for culture solution was employed in which plant roots treated with RI were pushed down gently with a mesh flange to bring all the root tissue into contact at the bottom. From outside the bottom of the container, an IP was placed for a time to take the root image. The IP could be replaced periodically to acquire successive RI distribution images of the root. Figure 4.4 is an example of a rice plant where 0.4 GBq/50 mL of ^{38}K (half-life: 7.6 min) was supplied to the plant. An IP was placed outside the container for 20 min to acquire the root image from the tip to the upper part. Tracing the root image allowed the amount of ^{38}K in the root to be calculated from the darkness of the image.

Fig. 4.4 An example of taking an image of ^{38}K absorption of rice roots. The rice roots supplied with ^{38}K in the culture solution were gently downed with a mesh board to the bottom of the container. An IP was attached at the outside of the bottom part of the container to acquire the radiation image of the roots

Similar usage of an IP was introduced in Part I, Chap. 2, Sects. 2.3.1 and 2.3.2, where an IP was placed close to a plant supplied with ^{15}O -water, and by changing the IP, another image of ^{15}O -water distribution was acquired. The difference between the images showed water movement.

4.2 Development of a Macroscopic Real-Time RI Imaging System (RRIS)

4.2.1 Construction of RRIS (First Generation)

Using an IP, live images of a living plant could be acquired by changing the IP successively; however, adjusting the positions of the images in different IPs was difficult, and it was not possible to trace the fast movement of elements. Therefore, a real-time imaging system was developed that allowed successive images to be taken and allowed the imaging of commercially available, conventional RIs. First, we adjusted all the devices with ^{32}P , whose β -ray energy is relatively high (1709 keV), offering the advantage of high-efficiency scintillator conversion. From the plant physiological point of view, phosphate is an important component of nucleic acids, and phospholipids play an important role in energy transformation. It is also known

Fig. 4.5 Principle of the real-time RI imaging system [2]. The radiation emitted from the sample was converted to light by a scintillator deposited on a fiber optic plate (FOP), and the light was detected by a highly sensitive single-photon counting camera to produce a radioactivity image

that phosphate supports photosynthesis as a substrate for the reaction and mediates signal transmission, etc.

The system we developed consisted of the following two steps: (1) conversion of β -rays emitted from plants into light by a scintillator and (2) detection of the light by a highly sensitive, single-photon-counting camera. The details of the imaging process were as follows. The β -rays from RI were converted to light by a scintillator; however, the intensity of the light was very low, and therefore, amplification of the light signal was needed. The light was amplified by an image intensifier unit (with GaAsP semiconductors in a photocathode), where light was converted to electrons in the photocathode, followed by amplification within a microchannel plate (MCP). The MCP consists of many thin channels of glass (capillary) to amplify electrons. When electrons were accelerated in an electric field by high voltage and introduced to capillaries, the electrons clash with the opposite side to emit more electrons. After several repetitions of these processes, an image was produced on the fluorescent surface, which was prepared on the MCP. The diameter of the capillary was $6\text{ }\mu\text{m}\phi$, which was the smallest size of MCP available now; however, this size was a key factor limiting the resolution level of the image. An image produced by electrons on a fluorescent surface was detected by a CCD camera in the AQUACOSMOS/VIM system (VIM system). Thirty image frames, consisting of 350,000 pixels/frame, were acquired per second, and the image was integrated for 1–3 min.

The area of the image covered $5.0 \times 7.0\text{ cm}$ of the plant sample. The measurement had to be performed in a dark box, since the light intensity from the sample was very weak even for a highly sensitive cooled CCD camera for single photon counting. This type of highly sensitive camera is severely damaged when environmental light leaks into the dark box. Therefore, first, the whole imaging system including the sample was kept under completely dark conditions. The sample was kept horizontal to acquire the plant image in the first generation of the imaging

Fig. 4.6 Overall image of the real-time RI imaging system (first generation). To prevent the damage of the light to a CCD camera, all imaging processes were performed in a *dark box*

Fig. 4.7 Schematic illustration of the real-time RI imaging system. To prevent the light from irradiating the plant, the FOP was covered with an Al foil. The β -ray penetrating the Al foil was converted to light by a scintillator and amplified by a GaAsP intensifier. Then, the light taken by the CCD camera was accumulated and produced the radiation image

system (Fig. 4.5). In the second and third generation of the imaging system, the sample was always kept vertical during imaging (Figs. 4.6 and 4.7).

4.2.2 Performance of RRIS

4.2.2.1 Dynamic Range of the System

The important parameters for the performance of the imaging system are sensitivity, resolution, and quantitative treatment of the image. However, considering the RIs to be applied, the performances are dependent on the energy and kind of radiation emitted from each nuclide. Seven nuclides, ^{14}C , ^{22}Na , ^{28}Mg , ^{65}Zn , ^{86}Rb , ^{109}Ca , and ^{137}Cs , were chosen to study the performance of the RRIS. Table 4.1 shows the features of the representative RIs used in the experiment. In the case of ^{28}Mg (half-life: 20.9 h), a radioactive equilibrium is attained with the daughter nuclide ^{28}Al (half-life: 2.2 min), whose energy is higher than that of ^{28}Mg . Therefore, the major contribution of the β -ray energy to imaging is considered to be derived from ^{28}Al .

To evaluate the performance of RRIS, a standard solution was prepared. Two to 3 μL of each standard solution was mounted on a polyethylene terephthalate sheet and covered with a polyethylene sheet (10 μm in thickness) after the solution was completely dried. In the case of ^{14}C , polyphenylene sulfide (1.2 μm in thickness) was used to cover the sheet. Then, the image acquired by RRIS was compared with that of an IP.

Table 4.2 shows the dynamic range of quantitative analysis in both RRIS and IP imaging measurements, with the lower limit and upper limit of the radiation counting. As shown in the Table, these properties were highly dependent on the kind of nuclide. To explain the lower limit of detection, ^{28}Mg measurement can be described as follows, as an example. In the case of RRIS, the lower limit for quantitative counting did not change with increasing accumulation time. The camera used in RRIS detected 1 photon per frame in 1 pixel at a speed of 30 frames/s. However, because a certain amount of noise exists at each count, such as dark current, the signal-to-noise ratio (S/N) does not improve with increasing

Table 4.1 Feature of the applicable nuclides for the RRIS

Nuclide	Decay	Half-life	β -ray energy (keV)		γ - or X-ray energy (keV)
			av.	max.	
C-14	β^-	5700 years	49.5	157	–
Na-22	β^+ , EC	2.6 years	216	546	1275, 551 (annihilation)
Mg-28	β^-	20.9 h	152	860	1589
(Al-28)	β^-	2.2 months	1242	2863	1779
P-32	β^-	14.3 days	695	1711	–
S-35	β^-	87.5 days	48.7	167	–
Ca-45	β^-	163 days	77.2	257	–
Mn-54	EC	312 years	–	–	835, 5.37 (Cr-K α)
Zn-65	β^+ , EC	244 days	143	329	1116, 551 (annihilation)
Rb-86	β^-	18.6 days	668	1774	1077
Cd-109	EC	461 days	–	–	22 (ae-K α), 88 (^{109}mAg)
Cs-137	β^-	30.2 years	514	1176	662 ($^{137\text{mBa}}$)

Table 4.2 Dynamic range of RRIS for the quantitative analysis

	RRIS				IP			
	The lower limit (Bq/mm ²)	The upper limit (Bq/mm ²)	Dynamic range	R-squared	The lower limit (Bq/mm ²)	The upper limit (Bq/mm ²)	Dynamic range	R-squared
<i>C-14</i> (min)								
3	4×10^0	2×10^3	3×10^2	0.9972	4×10^3	4×10^3	1×10^3	0.9999
5	2×10^0	2×10^3	1×10^3	0.9976	2×10^0	4×10^3	2×10^3	0.9997
10	2×10^0	2×10^3	1×10^3	0.9981	1×10^0	4×10^3	4×10^3	0.9996
15	2×10^0	2×10^3	1×10^3	0.9983	1×10^0	2×10^3	1×10^3	0.9951
<i>Na-22</i> (min)								
3	3×10^{-1}	3×10^2	1×10^3	0.9973	5×10^{-1}	6×10^2	1×10^3	0.9989
5	3×10^{-1}	3×10^2	1×10^3	0.9972	5×10^{-1}	6×10^2	1×10^3	0.9982
10	3×10^{-1}	3×10^2	1×10^3	0.9972	3×10^{-1}	6×10^2	2×10^3	0.9972
15	3×10^{-1}	3×10^2	1×10^3	0.9972	3×10^{-1}	6×10^2	2×10^3	0.9998
<i>Mg-28</i> (min)								
3	3×10^{-1}	1×10^2	5×10^2	0.9993	6×10^{-1}	3×10^2	5×10^2	0.9966
5	1×10^{-1}	1×10^2	1×10^3	0.9995	3×10^{-1}	3×10^2	1×10^3	0.9975
10	1×10^{-1}	1×10^2	1×10^3	0.9995	3×10^{-1}	3×10^2	1×10^3	0.9985
15	1×10^{-1}	1×10^2	1×10^3	0.9989	1×10^{-1}	3×10^2	2×10^3	0.9975
<i>Zn-65</i> (min)								
3	2×10^1	4×10^3	3×10^2	0.9992	2×10^1	2×10^4	1×10^3	0.9996
5	9×10^0	4×10^3	5×10^2	0.9990	2×10^1	2×10^4	1×10^3	0.9986
10	9×10^0	4×10^3	5×10^2	0.9990	9×10^0	9×10^3	1×10^3	0.9990
15	4×10^0	4×10^3	1×10^3	0.9990	9×10^0	9×10^3	1×10^3	0.9990
<i>Rb-86</i> (min)								
3	5×10^{-1}	3×10^2	5×10^2	0.9957	1×10^0	6×10^2	5×10^2	0.9994
5	3×10^{-1}	3×10^2	1×10^3	0.9959	5×10^{-1}	6×10^2	1×10^3	0.9996
10	3×10^{-1}	3×10^2	1×10^3	0.9956	3×10^{-1}	6×10^2	2×10^3	0.9996
15	1×10^{-1}	3×10^2	1×10^3	0.9960	3×10^{-1}	3×10^2	1×10^3	0.9968
<i>Cd-109</i> (min)								
3	2×10^0	1×10^3	5×10^2	0.9999	4×10^0	2×10^3	5×10^2	0.9997
5	1×10^0	1×10^3	1×10^3	1.000	2×10^0	2×10^3	1×10^3	0.9997
10	5×10^{-1}	1×10^3	2×10^3	0.9999	2×10^0	1×10^3	5×10^2	0.9999
15	5×10^{-1}	1×10^3	2×10^3	0.9999	1×10^0	1×10^3	1×10^3	0.9984
<i>Cs-137</i> (min)								
3	5×10^{-1}	3×10^2	5×10^2	0.9964	5×10^{-1}	1×10^2	2×10^3	0.9979
5	3×10^{-1}	3×10^2	1×10^3	0.9983	5×10^{-1}	3×10^2	5×10^2	0.9996
10	3×10^{-1}	3×10^2	1×10^3	0.9988	5×10^{-1}	6×10^2	1×10^3	0.9950
15	3×10^{-1}	3×10^2	1×10^3	0.9989	3×10^{-1}	3×10^2	1×10^3	0.9999

L.L. and U.L.: Lower limit and upper limit of the quantitative radiation counting [3] modified

accumulation time, which resulted in a plateau in the count after 5–15 min of accumulation (Fig. 4.8a, b). This tendency was observed for most of the other nuclides. In the case of an IP, the image intensity is dependent on the accumulation

Fig. 4.8 Quantitative detection limit in RRIS and IP [3]. Effect of an accumulation time in RRIS and an exposure time in IP on the quantitative detection limit using the ^{28}Mg standard solution. (a) and (b) S/N ratio and detection limit for RRIS, respectively. Similarly, (c) and (d) are for an IP. The quantitative detection limit was calculated as $\sqrt{2} \times 10 \times \sigma$ (standard deviation of the background value)

of radiation energy in the photostimulable phosphor. Therefore, with increasing exposure time, the intensity of the image increased, and the S/N ratio improved (Fig. 4.8c, d).

When the upper limit of the counting is taken into account, the value in the RRIS did not change with the accumulation time, since there is an upper count limit per frame in the camera. For short-term counting, 3 or 5 min, there was no upper counting limit detected for the IP, suggesting that the upper counting limit was higher for an IP than for the RRIS. In contrast, for long-term accumulation, there was an upper limit for detection in an IP, suggesting that there was a limit to the amount of radiation that could accumulate in the photostimulable phosphor.

The results showed that the dynamic range of the RRIS was on the order of 10^3 , suggesting that quantitative counting was possible even if the accumulation time was decreased from 15 to 3 or 5 min.

4.2.2.2 Distance Between FOS and the Plant

To acquire high image resolution, the distance between the plant sample and the FOS, on which the scintillator was deposited, should be as short as possible [4]. This distance will affect the quantitative analysis of the image. However, plants grow during imaging, which sometimes adds space between FOS and the sample. Therefore, evaluation should be performed to determine the relation between this distance and the intensity of the counting.

Six standard solutions of the nuclides ^{22}Na , ^{28}Mg , ^{65}Zn , ^{86}Rb , ^{109}Cd , and ^{137}Cs were prepared as point sources (3.3 mm^2), which were placed 0, 0.2, 0.3, and 0.4 mm from the surface of the FOS, and the radioactivity was counted. When the spot was 0.4 mm from the FOS surface, the counts decreased to 30–50% in all cases. One of the reasons for the decreasing count could be that the ROI (region of interest) in the image was too small to cover the radiation, which was spatially expanded. Therefore, when the area of the ROI increased from 3.3 to 51 mm^2 (in the case of ^{28}Mg , from 5.3 to 46 mm^2), the counting was not dependent on the distance between the FOS and the standard sample (Fig. 4.9). The increased area of the ROI was set so that the neighboring standard did not cover each expansion of the radiation.

4.2.2.3 Self-Absorption

With a decrease in the β -ray energy, the self-absorption rate of the β -ray increases, which results in a decrease in the signal intensity in the image. Since the degree of self-absorption depends on the kind of tissue, tissues of *Arabidopsis* were used to measure the decrease in intensity. To investigate the self-absorption, 7 nuclides were applied to the *Arabidopsis* tissue. In the case of ^{14}C , $^{14}\text{CO}_2$ produced by mixing ^{14}C -sodium hydrogen carbonate and lactic acid was supplied to the plant for 24 h. For the other nuclides, 3, 3, 10, 3, 10, and 0.5 kBq/mL of ^{22}Na , ^{28}Mg , ^{65}Zn , ^{86}Rb , ^{109}Cd , and ^{137}Cs , respectively, were supplied in culture solution and were grown for 3 days. After the treatment, flower parts, including the bulb and shoot tip, silique, stem, rosette leaf, and cauline leaf, were separated from the plant, and the intensity in the images acquired by the RRIS and radiation counts were compared. The radioactivity in each tissue was measured by a liquid scintillation counter for ^{14}C and by a γ -counter for the other nuclides.

Of the nuclides tested, the self-absorption effects for ^{28}Mg are shown as an example (Fig. 4.10). As shown in the figure, there was a good correlation between the image counts taken by RRIS and by a γ -counter in all the tissues investigated, where R^2 was more than 0.9. Among the other nuclides, ^{22}Na , ^{65}Zn , ^{86}Rb , ^{109}Cd , and ^{137}Cs also showed good correlations (Table 4.3). Since the β -ray energy of ^{14}C is low compared to that of the other nuclides employed, the relative intensity of the radioactivity in the tissue decreased drastically with increasing thickness of the tissue, except in the thin tissues. Although the precise amount was unable to be calculated, the radioactivity of thin tissues such as flowers or rosette leaves was

Fig. 4.9 Effect of the distance between FOS and a sample on the radiation measurement [3]. (a) Schematic illustration of the sample. Six standard radioisotope spots (^{22}Na , ^{28}Mg , ^{65}Zn , ^{86}Rb , ^{109}Cd , and ^{137}Cs) were prepared on a polyethylene terephthalate (PET) sheet and covered with a polyethylene (PE) sheet. On this sample, polycarbonate (PC) sheets (0.2, 0.3, and 0.4 mm in thickness) with holes were placed to maintain the distance between FOS and RI. (b) The ROI (region of interest) was set to be the same size as the standard RI spot (3.3 mm²; except for ^{28}Mg : 5.3 mm²). (c) The ROI was expanded to the maximum size to avoid superposing with the neighboring spots (51 mm²; except for ^{28}Mg : 46 mm²)

estimated with some errors permitted. When the correlation of ^{14}C was investigated, between the image counts acquired by RRIS and the radioactivity measurement by an IP, the result showed a high correlation, with an R^2 of 0.9857, suggesting that the contribution of the self-absorption rate of ^{14}C was the same for both measurements (Fig. 4.11). However, considering the low β -ray energy of ^{14}C , the performance of the RRIS should be taken into account carefully for ^{14}C imaging. This performance is described in more detail in the next Chapter, where the imaging of $^{14}\text{CO}_2$ gas fixation is presented.

In all of the other cases of the elements tested, the correlation of the image between the RRIS and an IP was high. Figure 4.12 shows actual images of Arabidopsis tissues acquired both by the RRIS and by an IP. As shown in the figure, the two images are almost the same, suggesting a high correlation.

Fig. 4.10 Effect of self-absorption on the ^{28}Mg measurement [3]. The relation between the intensity of the image acquired by RRIS and the radioactivity measured by a γ -counter was plotted for the ^{28}Mg radioactivity absorbed in plant samples. (a) whole plant; (b) flower; (c) silique; (d) stem; (e) rosette leaf; (f) cauline leaf. The accumulation time for RRIS was 15 min, and the exposure time of the IP was 60 min

4.2.2.4 Simulation of Self-Absorption

Although the rate of self-absorption was found to be dependent on the kind and energy level of the radiation, there are several factors that induce self-absorption. One of them is the performance of FOS for each nuclide, since several kinds of radiation are emitted from each nuclide. To determine what kind of radiation contributes to self-absorption, a simulation was carried out. A Monte Carlo simulation code, EGS5, which is used in simulating medical exposure, was modified, and practical scintillator detection of FOS according to the kind of radiation was calculated. As shown in Table 4.4, the major contributions to the detection were β -rays, positrons, and X-rays, and the detection efficiency of γ -rays was small since γ -rays penetrate the scintillator.

Table 4.3 Correlation between the counting of the image taken by RRIS and radioactivity counting in tissues

	C-14	Na-22	Mg-28	Zn-65	Rb-86	Cd-109	Cs-137
<i>Whole</i>							
<i>R²</i>	0.8396	0.9803	0.9869	0.9883	0.9659	0.9960	0.9937
<i>Slope</i>	1.006	10.10	27.27	2.342	83.77	2.083	5.579
<i>n</i>	111	90	109	76	90	101	117
<i>Flower</i>							
<i>R²</i>	0.9815	0.9699	0.9781	0.9280	0.9196	0.9835	0.9269
<i>Slope</i>	0.6200	7.410	19.58	2.270	83.27	1.842	4.467
<i>n</i>	7	14	15	13	11	13	19
<i>Silique</i>							
<i>R²</i>	0.7101	0.7088	0.9172	0.7504	0.8035	0.9863	0.9652
<i>Slope</i>	0.6830	6.898	19.46	2.014	68.80	1.888	4.695
<i>n</i>	14	24	27	24	24	24	39
<i>Stem</i>							
<i>R²</i>	0.2263	0.9625	0.9773	0.9377	0.8535	0.8996	0.8249
<i>Slope</i>	0.8768	5.930	20.37	1.614	68.36	1.418	3.820
<i>n</i>	43	24	28	8	22	24	33
<i>Rosette leaf</i>							
<i>R²</i>	0.8973	0.9964	0.9970	0.9932	0.9793	0.9971	0.9991
<i>Slope</i>	1.158	10.49	27.86	2.380	89.87	2.123	5.694
<i>n</i>	31	9	23	13	16	22	7
<i>Cauline leaf</i>							
<i>R²</i>	0.6384	0.9520	0.9903	0.9638	0.9929	0.9887	0.9978
<i>Slope</i>	0.7808	9.458	29.83	2.308	80.39	1.987	5.602
<i>n</i>	16	19	16	18	17	18	19

Fig. 4.11 Relation between the measurements of RRIS and IP [3]. After acquiring the image by RRIS (15 min), we measured the radioactivity of the standard sample prepared from ¹⁴C-sucrose by a γ-counter for 2 min

Fig. 4.12 Images of various *Arabidopsis* tissues containing RIs [4] modified. (a) Typical examples of tissue visualized by RRIS (upper) and IP (lower); from left to right: flower, silique, internode, rosette leaf, and cauline leaf. (i): ^{22}Na ; (ii): ^{65}Zn ; (iii): ^{86}Rb ; (iv): ^{109}Cd ; (v): ^{137}Cs . Pseudocolors were assigned according to the intensity/ mm^2 for RRIS and PSL/ mm^2 for an IP in the image. (b) Signal intensities detected in tissues were plotted against the radioactivity determined by the gamma-counting method

Table 4.4 Type of radiation detected by FOS by simulation [3]

	Radiation	Average kinetic energy (MeV)	Energy absorbed by the CsI (MeV/100,000decay)	Contribution percentage (%)
C-14	β	0.049	39.3	100.0
Na-22	Positron	0.216	1709.7	93.9
	γ	1.275	72.4	4.0
	Ce Total	1.274	0.0	0.0
Mg-28	β	0.152	1004.0	64.0
	γ_1	0.031	446.0	28.6
	γ_7	1.373	39.7	2.6
	γ_4	0.942	32.9	2.1
	γ_2	0.401	31.4	2.0
	γ_9	1.620	3.2	0.2
	γ_8	1.589	2.5	0.0
	γ_{10}	1.014	0.5	0.0
	Ce (K, γ_1)	0.029	0.0	0.0
	Anger (K)	0.001	0.0	0.0
	Ce (L, γ_1)	0.031	0.0	0.0
	γ_3	0.607	0.0	0.0
	γ_5	0.983	0.0	0.0
	γ_6	1.342	0.0	0.0
	X-ray (K)	1.487	0.0	0.0
Al-28	β	1.242	5030.8	98.0
	γ	1.779	73.7	1.4
Zn-65	X-ray ($K\alpha_1$)	0.008	44.3	38.9
	X-ray ($K\alpha_2$)	0.008	22.2	18.4
	γ_3	1.116	32.8	27.2
	X-ray ($K\beta$)	0.009	11.5	9.0
	Positron	0.143	9.0	7.5
	ce (K, γ_3)	1.107	0.3	0.3
	Ce (L, γ_3)	1.114	0.0	0.0
	γ_2	0.771	0.0	0.0
	γ_1	0.345	0.0	0.0
	Anger (K)	0.007	0.0	0.0
	X-ray (L)	0.001	0.0	0.0
	Anger (L)	0.001	0.0	0.0
Rb-86	β	0.668	4115.3	99.8
	γ	1.077	7.6	0.2
	X-ray ($K\alpha_1$)	0.013	0.0	0.0
	X-ray ($K\alpha_2$)	0.013	0.0	0.0
	Anger (K)	0.011	0.0	0.0
	Anger (L)	0.002	0.0	0.0
Cd-109	X-ray ($K\alpha_1$)	0.022	394.4	48.4
	X-ray ($K\alpha_2$)	0.022	210.4	25.8
	X-ray ($K\beta$)	0.025	131.0	16.1

(continued)

Table 4.4 (continued)

	Radiation	Average kinetic energy (MeV)	Energy absorbed by the CsI (MeV/100,000decay)	Contribution percentage (%)
	Ce (L)	0.084	29.5	2.6
	γ	0.088	27.9	3.4
	Ce (K)	0.063	8.0	1.0
	Ce (M)	0.087	6.4	0.8
	X-ray (L)	0.003	0.4	0.0
	Anger (K)	0.019	0.0	0.0
	Anger (L)	0.003	0.0	0.0
Cs-137	β	0.188	1472.2	69.2
	Ce (K, γ_2)	0.624	443.6	20.8
	Ce (L, γ_2)	0.656	77.3	3.6
	γ_2	0.662	65.9	3.1
	X-ray ($K\alpha_1$)	0.032	24.7	1.2
	Ce (M, γ_2)	0.660	16.5	0.8
	X-ray ($K\alpha_2$)	0.032	12.7	0.6
	X-ray ($K\beta$)	0.036	11.9	0.6
	Ce (N, γ_2)	0.661	3.8	0.2
	X-ray (L)	0.004	0.2	0.0
	γ_1	0.284	0.0	0.0
	Anger (K)	0.026	0.0	0.0
	Anger (L)	0.004	0.0	0.0

The other factor for self-absorption is the distribution of the nuclide within the tissue, which is not spatially uniform. To study the self-absorption derived from different distribution patterns of the nuclide within the plant tissue, a pipe model mimicking the stem was used, where the nuclide was distributed at the surface, interior, and center of the pipe (Fig. 4.13).

In the cases of ^{28}Al , ^{65}Zn , ^{86}Rb , and ^{109}Cd , there was no great difference in counting due to the distribution of the nuclide. In the cases of ^{22}Na , ^{28}Mg , and ^{137}Cs , the difference in the distribution affected the counting only of the nuclides distributed in the center. However, the vascular bundle, where nuclides are estimated to accumulate, is located relatively close to the surface, and it is not likely that the nuclides are present in the center of the stem. In the case of ^{14}C , there was a large difference in the effect of the distribution on the counting, especially between the homogeneous distribution and the localized distributions localized at the center or at the surface. Therefore, to apply ^{14}C for imaging, self-absorption must be taken into consideration. However, when the counting area was fixed and the change at the same site of the image with time was compared, numerical treatment of the change in the counting at this area was possible, as in the relative analysis.

The efficiency of producing the image was the integrated result of the various kinds of radiation emitted from the nuclide and its energy, specific to each nuclide.

Fig. 4.13 Simulation of the potential impact of steric distribution changes of RI [4]. To estimate the contribution of each radiation type from various RIs to RRIS imaging, cylindrical plant phantoms (2 mm in diameter) were prepared to perform the EGS5 simulation. The energy deposited in the Cs (Tl)I scintillator was calculated for each individual ray, and the total energy absorbed by the scintillator and contribution percentages of each radiation type were determined for each phantom. **(a)** Designed phantom; **(b)** imparted energy relative to ^{22}Na at the center

Generally, when the β -ray energy is high, the self-absorption is low, and the reverse effect occurs with low β -ray energy. In the case of X-rays, self-absorption is low, and the radiation penetrates the plant tissue but not the scintillator. High-energy γ -rays result in low counting efficiency since they penetrate both the plant tissue and the scintillator. With these results, it was possible to estimate the counting efficiency of other nuclides, for example, ^{32}P , which has a β -ray energy level similar to that of ^{86}Rb .

4.2.2.5 Actual Image of the Plant Sample

To acquire the plant image, a soybean plant (*Glycine max.* cv. Enley) was grown for 2 weeks, and then, Hoagland solution containing ^{32}P (P: 10 μM , 5 MBq/100 mL) was supplied for 24 h. Then, the ^{32}P image of the third trifoliate leaf was taken by RRIS. Figure 4.14 compares the images acquired by a prototype RRIS, as shown in Fig. 4.7, and an IP. It was found that the vein could be detected in a distinctly shorter time, a few seconds, than that needed for an IP, a few minutes.

Since the β -ray energy of ^{32}P is relatively high (max. energy: 1.7 MeV), another nuclide with lower β -ray energy, ^{45}Ca (max. energy: 0.257 MeV) was also used to compare the sensitivity of the imaging system. Figure 4.15 shows successive images of ^{45}Ca when this nuclide was supplied to the root of the soybean plant. The ^{45}Ca

Fig. 4.14 Comparison of the ^{32}P images acquired by RRIS and an IP [5]. ^{32}P images of a center leaf of the first trifoliolate leaves of a soybean plant are shown after the treatment with ^{32}P for 24 h. Two images of the leaf at each imaging condition are shown. RI images acquired by the RRIS (*upper*) and an IP (*lower*) are presented. The ^{32}P image in a leaf can detect as early as 10 s by the RRIS

image in the third trifoliolate leaf was taken after 1, 5, and 15 min by an IP (A) and after 10 s, 1 and 5 min by our imaging system (B). From the image series, it was found that image accumulation for 1 min by the RRIS provided a similar image to IP exposure for 15 min.

The results obtained by both nuclides indicated that the sensitivity of our imaging system was approximately 10 times higher than that of IP. The shorter accumulation

Fig. 4.15 Comparison of the ^{45}Ca images acquired by the RRIS and an IP [6]. The images of ^{45}Ca in trifoliate leaves by the RRIS (a) and IP (b). Veins are clearly observed in both imaging methods. The sensitivity of RRIS is higher than that of an IP, similar to those of ^{32}P images in Fig. 4.14

time for the RRIS indicates that a series of successive shorter images enables the production of a movie showing the real-time movement of the ions.

4.2.3 Imaging by Prototype Imaging System

4.2.3.1 ^{32}P Imaging in a Soybean Plant

Using the prototype imaging system, the behavior of ion uptake from the roots was shown. The first trial was ^{32}P -phosphate absorption in a soybean plant (*Glycine max.* cv. Enley). A soybean plant was grown for 2 and 6 weeks and then harvested to acquire leaf and young pod images, respectively. After ^{32}P (orthophosphate containing approximately 600 kBq/mL ^{32}P) solution was supplied from the root, several kinds of tissues from the whole plant were selected for imaging: meristem with young leaves, young trifoliate, center leaf of an expanded trifoliate, first leaf, and a young peapod.

Figure 4.16 is an image of the four tissues. In the leaves, it was expected that a high amount of ^{32}P -phosphate would be shown in the vein, since phosphate is transferred through the vein after absorption from roots. This was true in the first leaf. However, in contrast, high accumulation of phosphate was found between the veins in the first trifoliate leaf, suggesting a different route to transfer phosphate or leaking from the vein in the trifoliate leaf. A large amount of water was found to leak from xylem tissue in an internode (see Part I, Chap. 2, Sect. 2.3), and it was estimated to replace the water already present. In the case of phosphate, even if there was an accumulation of phosphate between the veins, there could not be any reason to

Fig. 4.16 ^{32}P -phosphate distribution in a soybean plant [7]. ^{32}P -phosphate was supplied from the root, and the accumulation pattern of ^{32}P was recorded by RRIS. ^{32}P -phosphate was first moved up to the youngest tissue and subsequently to the relatively older tissue. In one leaf, ^{32}P was found to be highly accumulated between the veins and shown as dots (*bottom left image*). The successive images with lap time are shown in Fig. 4.17

replace the phosphate already there. It was not known when and how phosphate leaked from the vein and why there was a different accumulation pattern between the first leaf and the first trifoliate leaf.

The successive images showed a rapid and high accumulation of ^{32}P in the youngest tissue, indicating that the absorbed phosphate was transferred primarily to the youngest tissue and then, when the ^{32}P in the youngest tissue was saturated, provided to the next younger tissues (Fig. 4.17). It seemed that ^{32}P -phosphate supply was prioritized according to the age of the tissue. This movement was obtained as a movie, and the lap time images are shown in Fig. 4.17.

Figure 4.18 shows how ^{32}P is transferred within the pod when supplied from the root. Since the image is based on radiation counting, the amount of ^{32}P -phosphate in the image could be analyzed. When the radioactivity in a specific area (marked as circle) was removed from the successive images, the behavior of the phosphate increase could be analyzed. Most of the ^{32}P -phosphate transferred in the direction of the pod initially accumulated at the bottom part of the pod during the first 2 h. Then, ^{32}P was transferred to the two seeds within the pod, not to the surface of the pod.

Fig. 4.17 ^{32}P translocation images in a soybean plant [8]. (a) meristem with young leaves; (b) young trifoliate; (c) center leaf of an expanded trifoliate; (d) young peapod. (a) 3-min integrated image, (b), (c), and (d) 4 min integrated image. White bar: 10 mm

When the accumulation of ^{32}P between the seeds was compared, the accumulation rate and amount in the two seeds were approximately the same.

4.2.3.2 ^{14}C Imaging in a Rice Plant

In this section, the visualization of ^{14}C -labeled amino acid absorption in a rice plant using the prototype RRIS is presented. The beginning of this research was as follows. Recently, organic farming, without depending on chemical fertilizer, has

Fig. 4.18 Image analysis of the ^{32}P -phosphate accumulation in the pod [7]. The pseudocolor was assigned based on the counts in the pixels. First, ^{32}P accumulated at the bottom part of the pod and subsequently transferred to the other parts of the pod. There was no difference in the transfer speed or amount of ^{32}P between the two seeds

become popular, where incompletely decomposed organic matter, including plants, is supplied. However, it has been taken for granted that plants live on 17 inorganic ions and that other chemicals are not needed for growth. If plants can absorb amino acids, along with inorganic ions, this evidence could provide scientific support for organic farming from some perspectives. Therefore, the rice plant (*Oryza sativa* L. Nihonbare) was selected to investigate whether it could absorb amino acids and to analyze the chemical forms of the amino acids within the plant. The imaging part of this study, visualization of amino acid absorption, is shown below with some results obtained from chemical analysis.

Fig. 4.19 ^{14}C -glutamine absorption images of the roots from the solution [9]. Twenty milliliters of ^{14}C -glutamine (18.5 kB/mL) solution were supplied to a two-day seedling of the rice plant, and the ^{14}C accumulation images of the root were monitored for 30 h. The accumulation time for each image was 10 min

First, by applying doubly labeled (^{15}N and ^{13}C) glutamine to 6-day-old seedlings, ^{15}N - and ^{13}C -glutamine were detected in both the underground and aboveground parts of the plant by a high-performance liquid chromatography equipped with an ion trap mass spectrometer. The results suggested that glutamine itself was absorbed from the root without decomposition in the rhizosphere and was transferred to the aboveground part.

Then, real-time imaging of glutamine absorption from roots was performed to analyze the uptake of amino acids in roots. The container with root was placed on a Cs(Tl)I scintillator that was covered with polystyrene film (4 μm in thickness). Then, by pushing the root gently with a sponge, the root was set close to the scintillator. Approximately 20 mL of ^{14}C -glutamine solution (18.5 kB/mL, containing 250 μM N) was supplied to the container, and the uptake of the amino acids in the roots was visualized (Fig. 4.19). The image was integrated every 10 min/frame until 43 h. When the successive images were produced as a movie, it was interesting to note that first, a ^{14}C -glutamine accumulation front appeared around the lower part of the roots in the solution, and then, the ^{14}C -glutamine abruptly moved upward and was absorbed by the roots, indicating that there was no continuous absorption of the amino acid uptake but suggesting that optimal timing and optimal concentration were needed for the ion movement toward the root in solution.

Within the same root, the uptake activity and accumulation of glutamine were not uniform, and high accumulation of glutamine was always shown at the root tip. The absorbed amount in the root plateaued after approximately 10 h. When transpiration was prevented by covering the leaf surface with Vaseline, the absorption rate and

Fig. 4.20 Comparison of the ^{14}C -glutamine absorption manner of the root when Vaseline was pasted on the leaves [9]. ROIs (region of interest) were set at the root tip and center of the root to plot the ^{14}C -glutamine absorption speed. When Vaseline was applied to the leaves (a), the absorption amount and speed of ^{14}C -glutamine were lower than those of the control (b). The increase in ^{14}C -glutamine ceased at approximately 12 h, which suggests that most of the ^{14}C -glutamine supplied to the solution has been absorbed by the root by this time

accumulation amount of glutamine in roots decreased (Fig. 4.20), suggesting that the accumulation amount was dependent on transpiration activity. A similar result was obtained when valine or alanine was supplied to the rice plant from the root, but the amount of absorbed glutamine was much higher than those of valine or alanine (data not shown).

Further tracer work using doubly labeled (^{15}N and ^{13}C) glutamine and valine showed the difference in the availability of organic nutrients in a rice plant. When the absorption of glutamine and valine was compared, in the case of glutamine, the accumulation of ^{13}C in the plant was lower than the absorbed amount although the absorption and accumulation amounts of ^{15}N in the plant were the same. It was suggested that a portion of the amino acids assimilated into the plant was lost as carbon dioxide ($^{13}\text{CO}_2$) through respiration, and the assimilation of glutamine occurred more smoothly than that of valine.

4.2.4 Introduction of the Plant Irradiating System (Second Generation)

4.2.4.1 Introduction of a Plant Box

Since light irradiation on the aboveground part of the plant is needed during imaging, a plant box was prepared using aluminum plates and set in the prototype imaging

Fig. 4.21 Preparation of a plant box for RRIS imaging [10]. A plant box (200 × 300 × 50 mm) was prepared with a 5-mm-thick Al plate with a rectangle window of 100 × 100 mm. One hundred LEDs were installed in the box to irradiate the plant. All plates were sealed well with Al tape to prevent complete light leakage

box [6, 10–12]. The container was a rectangular box (50 mm × 200 mm × 300 mm). Figure 4.21 shows a picture of the plant container equipped with 100 light-emitting diodes (LEDs) capable of emitting $120 \mu\text{mol}/\text{m}^2 \text{ s}$ onto the sample plant. By completely covering the plant and the light source, the container prevented external light from producing noise during the imaging processes. Since the scintillator allows light penetration, its surface facing the container was covered with an aluminum sheet 50 μm in thickness. Under this condition, light emitted by the LEDs was completely shielded, and only the β -rays penetrating the aluminum plate reached the scintillator without noticeable deterioration. The size of the scintillator working surface on the FOP was 10 cm × 20 cm. The LEDs could be switched on and off. The conditions of day and night were prepared by turning the LEDs on and off to produce a 16 h L/8 h D light/dark cycle for the aboveground part of the plant, while the roots were kept continuously in the dark. To control the temperature and humidity in the box, airflow was introduced into the box from the upper part.

The first experiment using the prepared plant box examined ^{32}P -phosphate absorption in *Lotus japonicas* cv. Miyakogusa. Phosphate absorption images were taken after the application of ^{32}P -phosphate (1 MBq/30 mL Hoagland culture solution) to the plant, and images were accumulated every 3 min for 30 h. The successive absorption images of phosphate from root to the aboveground part are

Fig. 4.22 Successive ^{32}P -phosphate absorption images in lotus [6]. The plant after 25 days of germination was set in a plant box, as illustrated in Fig. 21. ^{32}P -phosphate solution was supplied, and absorption images were taken 30 h after the treatment. The accumulation time for each image was 3 min. The magnification image of the root tips after 25 h is shown in the *upper right*

shown in Fig. 4.22. After 30 min of the ^{32}P -phosphate supply, the ^{32}P signal had reached the top of the stem. In the case of the root, high accumulation of phosphate in root tips was always shown throughout the successive absorption images, suggesting that a high amount of phosphate was always required in actively proliferating tissue, similar to that of the N source shown in Fig. 4.19. A magnification of the root tip is also shown in the figure.

To determine the difference in phosphate accumulation among the tissues in the plant under different light conditions while the plant was grown under 16 h L/8 h D light/dark conditions, the absorption amount of ^{32}P -phosphate in different tissues during development was plotted. In the flowers, the absorption curve was indifferent to the light cycle, but in the leaves, the absorption curve became high in light and low in the dark, where the opposite tendency of the absorption curve was observed in the roots (Fig. 4.23).

An interesting thing to note regarding phosphate absorption was that the amount of phosphate transferred to younger tissue was always high; however, under phosphate-deficient conditions, the amount of phosphate transferred to elderly leaves was similar to that transferred to younger leaves. Another interesting finding was that

Fig. 4.23 ^{32}P -phosphate uptake from lotus roots. The plant sample was set in a plant box and grown under 16 h L/8 h D light/dark conditions. During 40 h, successive ^{32}P -phosphate uptake images were taken, and the ^{32}P signal amounts in 3 different tissues, shown as red circles, were removed and plotted. Purple columns in the figure show dark periods. The absorption curve shows that the flower parts were indifferent to the light cycle, and leaves absorbed high amounts of ^{32}P -phosphate during the light period, which was opposite to the behavior of the roots. The ^{32}P uptake was indifferent to the light cycle in flowers and increased during the light period in leaves but increased during the dark period in roots. The vertical axis shows the intensity of the counts

under phosphate-deficient conditions, morphological differences in root shape were induced, which decreased the amount of phosphate in the roots although there was no difference in root weight. The images obtained by the RRIS showed that it is a promising tool to trace ion transport, providing us with many new questions to be studied. This study was further developed to identify the kind and role of phosphate

transporter genes under different conditions and in different kinds of plants. In particular, this study was further developed to study the expression of 7 phosphate transporter genes, from *LjPT1* to *LjPT7*, and the expression of each transporter gene in different tissues at different developmental stages was investigated (data not shown). However, the details are omitted here.

4.2.5 Introduction of Dark Period while Acquiring the Image (Third Generation)

The first generation of the RRIS system was applicable only under light-free conditions to protect the highly sensitive charge-coupled device camera; therefore, the plant was not able to maintain suitable photosynthesis activities. The second generation of the RRIS system enabled experiments under light irradiation by incorporating a plant box. This system was able to detect both high-energy beta emitters (e.g., ^{32}P) and X-ray/ γ -ray emitters (e.g., ^{109}Cd). However, since the irradiated light was able to penetrate the scintillator and this light increased the background noise of the photon-counting camera, it was necessary to install an aluminum shield (50 μm in thickness) over the scintillator. Since β -rays from low-energy β -ray emitters (e.g., ^{14}C , ^{35}S , ^{45}Ca) were not able to pass through the aluminum shield, these radioisotopes were not detected by this system. Among the low-energy β -ray emitters, the detection of ^{14}C under light-irradiated conditions was required to study the movement of photosynthetic products. Other nuclides, S and Ca, are major essential elements for plants; therefore, ^{35}S and ^{45}Ca are also important radioisotopes to analyze. Consequently, the system was improved to detect low-energy β -ray emitters.

Figure 4.24 shows a comparison of the second RRIS and new third generation RRIS. Instead of shielding the photon-counting camera from the light-emitting diode (LED) light with an aluminum shield and inner light-tight box, the new system was able to turn off the LED lights during photon counting. The detailed explanation is that the lighting system was integrated with the photon-counting camera control system. The power to the LED light was shut off by using a PC-controllable relay that was controlled by the AQUACOSMOS photon-counting camera control system via RS-232C. With this integrated control system, even if the system was accidentally stopped at any step of the sequence, the photon-counting camera was not damaged by LED light. The clustered LED light was composed of 64 red and 6 blue LEDs. These 2 types of LEDs had suitable peak wavelengths for photosynthesis (660 and 470 nm). Plants are more sensitive to the beginning of night than to daybreak; therefore, the properties of light, such as wavelength, should be carefully considered. Another device was the ventilation of the light-tight box. Opaque tubes were connected to the light box, and the air in the box was circulated at the laboratory temperature, which was maintained in a suitable range for the plants.

Fig. 4.24 Comparison of the previous RRIS (second generation) and new RRIS (third generation) [13]

With this change, both the detection of low-energy β emitters and light irradiation of the plant could be performed. In addition, a commercially available digital camera was added to the system to acquire photographic images of the test plant (Figs. 4.25, 4.26). The camera was placed opposite the photon-counting camera and was operated by remote control. This change enabled the continuous comparison of photon-counting images and photographic images throughout the experimental period.

^{35}S -labeled sulfate was chosen as the radioisotope tracer to verify the new system's ability to detect weak β -rays. For the test experiment, a rice plant (*Oryza sativa* L. var. Nipponbare) was grown for 18 days, and then carrier-free ^{35}S was supplied to 30 mL of culture medium, which contained approximately 1 mM sulfate. The specific radioactivity of ^{35}S was 170 kBq/ μmol .

The cycle of intermittent lighting was set to 1 h, and the photon-counting time in each cycle was set to 15 min. Figure 4.27 shows the image of the ^{35}S absorbed in the third, fourth, and fifth leaves of the two rice plants. The intermittent lightning system maintained the plant in healthy condition for several days. The increasing intensity of ^{35}S in the images showed that the test plants continued to absorb sulfate for 72 h after the application. As shown in Fig. 4.27, the third leaf absorbed sulfate quickly, but the distribution of ^{35}S was limited to the base area of the leaf blade. In contrast, in the fourth leaf, ^{35}S tended to be distributed over the entire area of the leaf blade. By superposing the radiation image on the picture, it was found which part of the leaf accumulated ^{35}S . In the fifth leaf, ^{35}S was detected later than in the other leaves, but the final intensity of the signal was higher than in the others. However, the increase

Fig. 4.25 Overview of the new RRIS [13]

in the signal area in the fifth leaf does not show the specific accumulation site of ^{35}S within the leaf; the fifth leaf itself grew larger during the imaging, which could not be determined from the RRIS image alone.

A revised RRIS with the ability to trace low-energy β emitters, such as ^{14}C , ^{35}S , and ^{45}Ca , was developed, and the capability of the new RRIS was verified. In particular, an advantage of the new RRIS is the ability to superimpose time-course photon-counting images on the photographic images of plants simultaneously. With this third-generation RRIS, another goal emerged: to image ^{14}C -labeled carbon dioxide gas and ^{14}C -labeled metabolites for the practical study of photosynthesis. (See next chapter).

4.2.6 Large-Scale Plant Sample

4.2.6.1 Plastic Scintillator

In all the generations of RRIS developed, a Cs(Tl)I scintillator deposited to a fiber optic plate (FOS) was used to convert radiation into light. However, one unit size of the scintillator was fixed at $10\text{ cm} \times 10\text{ cm}$, which was too small to observe an entire

Fig. 4.26 Scintillator plates and plant arrangement of the new RRIS [13]

plant of larger size. To cover a large area of the sample, several FOSs were connected to each other. However, the plant samples sometimes grow much larger than the scintillator. For example, a rice plant can be as high as 50–60 cm, and it is difficult to prepare many expensive FOSs to cover the area of the whole plant.

To image large plants, six types of low-priced plastic scintillators were investigated: FOS, BC-400, BC-408 (Saint-Gobain, La Défense Cedex, France), and Lumineard-A, B, C, and D (Tokyo Printing Ink Mfg. Co., Ltd., Tokyo Japan). The thicknesses of the scintillators were as follows: FOS: 0.1 mm, BC-400: 0.5 mm, BC-408: 5 mm, Lumineard-A: 0.5 mm, Lumineard-B: 1 mm, Lumineard-C: 1.3 mm, and Lumineard-D: 5 mm. Lumineard-B and Lumineard-D were made from 2 and 10 sheets of Lumineard-A glued together, respectively. As an optical adhesive, a mixture of KE-103 and CAT-103 (Shin-Etsu Chemical Co., Ltd. Tokyo, Japan) at a ratio of 1:20 was used.

Then, the performance of the plastic scintillators was studied by preparing the standard solution. Two microliters of the ^{14}C -labeled sucrose solution (6–51,000 Bq/spot) were spotted on a polyethylene terephthalate (PET) sheet. The size of the spots was approximately 3.6 mm^2 , and the performance of these plastic scintillators, such as quantification, detection limit, and resolution, was studied and compared to that of FOS. Table 4.5 shows the properties of each scintillator.

The lower quantification limits in all scintillators (Bq/mm^2) were approximately constant, regardless of the integration time. Among the 6 plastic scintillators, the

Fig. 4.27 Sequences of successive images of test plants acquired using the new RRIS [13]. ^{35}S -sulfate (170 kBq/ μmol) was supplied to 30 mL of culture solution, and successive accumulation images of ^{35}S in two rice plants were taken for 72 h after the treatment. The upper sequence a shows ^{35}S images of ^{35}S -sulfate absorbed by the rice plant, and the pseudocolor indicates the signal intensity (red represents high intensity). The lower sequence b shows the superimposed image. Blue gradational images represent the ^{35}S image, and grayscale images represent photographic images that were processed using Sobel filter. By superposing the images, we found that the increase in ^{35}S in the fifth leaf was not due to a change in accumulation pattern but due to the growth of the leaf, which was not known only from ^{35}S images

Lumineard-C showed the lowest quantification limit. Since plastic scintillators are not completely transparent, optical refraction seemed to occur in the BC-400 and BC-408. The interaction between the Lumineard-A and the low-energy β -rays irradiated from ^{14}C seemed weak because the Lumineard-A had the lowest thickness of 0.5 mm. To improve the interaction efficiency, the Lumineard-B and Lumineard-D were prepared by folding several sheets of Lumineard. However, the light converted by the 5 mm-thick Lumineard-D spread while passing through the scintillator, resulting in a decrease in the signal intensity. Considering all the performances, the Lumineard-C was selected for imaging ^{14}C in a large sample. The ^{14}C images acquired by the plastic scintillator are presented in the next section, where $^{14}\text{CO}_2$ gas fixation images of a rice and a corn plant are shown.

In the case of ^{32}P imaging with a plastic scintillator, similar evaluation tests were performed using a ^{32}P spot (12.2 Bq/ cm^2). The resolution was obtained from the line profile of the spot. The FWHM became wider in the order A, B, C among the scintillators. Considering other results, the study demonstrated that the 1 mm thick

Table 4.5 Features of the plastic scintillators and FOS [14]

	Integration time (min)	The lower limit (Bq/mm ²)	The upper limit (Bq/mm ²)	R-squared	Light output relative to FOS (%)
FOS	3	1×10^0	2×10^1	0.9965	–
	5	1×10^0	2×10^1	0.9971	–
	10	6×10^{-1}	2×10^1	0.9980	–
	15	1×10^0	7×10^1	0.9991	–
BC-400	3	2×10^0	6×10^2	0.9944	38
	5	1×10^0	6×10^2	0.9947	37
	10	1×10^0	6×10^2	0.9943	35
	15	2×10^0	6×10^2	0.9947	33
BC-408	3	2×10^0	6×10^2	0.9915	31
	5	1×10^0	6×10^2	0.9925	31
	10	1×10^0	6×10^2	0.9937	30
	15	2×10^0	6×10^2	0.9938	27
Lumineard-A	3	1×10^0	6×10^4	0.9924	50
	5	1×10^0	6×10^2	0.9922	49
	10	1×10^0	6×10^2	0.9926	47
	15	2×10^0	6×10^2	0.9963	45
Lumineard-B	3	1×10^0	3×10^2	0.9987	66
	5	1×10^0	3×10^2	0.9988	65
	10	1×10^0	3×10^2	0.9987	63
	15	1×10^0	3×10^2	0.9980	57
Lumineard-C	3	3×10^{-1}	3×10^2	0.9933	56
	5	3×10^{-1}	3×10^2	0.9920	56
	10	3×10^{-1}	3×10^2	0.9919	52
	15	3×10^{-1}	3×10^2	0.9950	49
Lumineard-D	3	2×10^0	6×10^2	0.9993	33
	5	2×10^0	6×10^2	0.9991	32
	10	2×10^0	6×10^2	0.9991	31
	15	5×10^0	6×10^2	0.9990	28

Lumineard-B has the best performance among low-cost plastic scintillators and can visualize and quantify ^{32}P in the RRIS despite lower light output and resolution.

4.2.6.2 Images Obtained by a Plastic Scintillator

Using the Lumineard-B as a plastic scintillator for ^{32}P imaging, the uptake of ^{32}P -phosphate in a rice plant was visualized. A 27-day-old rice plant (*Oryza sativa*

Fig. 4.28 ^{32}P -phosphate absorption in a rice plant using a plastic scintillator, Lumineard-B [14]. (a) Photograph of the rice plant set on Lumineard-B. (b) Sequential ^{32}P images taken by RRIS. Scale bar: 10 cm. (c) Time course of ^{32}P signal intensity in each leaf. The leaves were chronologically defined as the top leaf, second leaf, third leaf, and fourth leaf beginning from the youngest leaf, as indicated in the photograph

L. Dongjin) was fixed on a Lumineard-B converter covered with 2 μm thick aluminum film. The size of the Lumineard-B was approximately 800 mm $\times$ 200 mm. After the plant was transferred to 40 mL of culture medium containing 15 MBq of ^{32}P -phosphate, continuous RRIS imaging was performed in the dark box for 24 h. Light was provided at intervals of 10 min, and images were taken during the dark period. The time-course movement of ^{32}P -phosphate in a rice plant was measured by setting ROIs at each leaf (Fig. 4.28). The leaves were assigned as the top leaf, second leaf, third leaf, and fourth leaf in chronological order from the youngest leaf. There was a drastic change in the signal intensity according to the age of the leaf, where the ^{32}P signal intensity was the highest in the youngest leaf, indicating a high requirement of phosphorus for growth. Furthermore, ^{32}P signal intensity increased monotonically in each leaf. However, the rate of increase declined gradually, possibly because phosphorus in the leaves was translocated to other younger tissues. This decline was remarkable, particularly in the third leaf at 7 h after the treatment.

4.2.7 Summary of RRIS Development

A real-time RI imaging system (RRIS) was developed, composed of a Cs(Tl)I scintillator deposited on a fiber optic plate (FOP) and a highly sensitive charge-coupled (CCD) camera with an image intensifier unit. The imaging system was developed in 3 steps to enable irradiation of the plant during visualization. With the third generation of the system, it was possible to visualize the uptake behavior of many kinds of nuclides, such as ^{14}C , ^{22}Na , ^{38}Mg , ^{45}Ca , ^{32}P , ^{65}Zn , ^{86}Rb , ^{109}Cd , and ^{137}Cs , in a plant. It was shown that even among the leaves or within a root, the routes of element transfer and the accumulation behavior were different.

The development of the imaging system is summarized as follows (Fig. 4.29):

1. First generation: The scintillator selected was Cs(Tl)I, which was deposited on a multichannel plate. Everything, including the plant sample, was set in a dark container.
2. Second generation: A plant box was prepared so that only the aboveground part received light irradiation. The FOS had to be covered with Al foil to prevent light penetration, which prevented the counting of low-energy β -rays.
3. Third generation: Weak radiation energy could be detected, such as ^{14}C or ^{35}S . The light was off when the CCD camera was working. A camera was set to take a picture on which the radiation image could be superposes. $^{14}\text{CO}_2$ gas was generated and supplied to the plant. The fixed gas in the plant could be imaged.

Although RRIS has been used to study element movement in plants, its use has been limited to small plants because of its small field of view (100×200 mm). Therefore, the RRIS has been further updated to image an RI in a large plant. The study demonstrated that 1 mm thick Lumineard-B and Lumineard-C had the best performance among low-cost plastic scintillators and could visualize and quantify ^{32}P and ^{14}C , respectively, in RRIS despite lower light output and resolution than those of FOS. As a result, we are now equipped to analyze phosphorus movement in larger plants for advanced growth stages. This updated RRIS has a field of view of approximately 500×600 mm.

4.3 Element Absorption from Roots

4.3.1 Water Culture and Soil Culture

4.3.1.1 ^{32}P -Phosphate Absorption in a Rice Plant

Since β -rays from ^{32}P (1.7 MeV) can penetrate soil, it was possible to use the RRIS to visualize how phosphate in soil can be absorbed by roots. To compare the soil culture, water culture was also performed, and phosphate uptake images were taken.

Fig. 4.29 Development of the real-time RI imaging system [7]. The sample for imaging and a scintillator device were kept in the dark in the first generation, since the CCD camera employed to image the light from the scintillator is highly sensitive to light. Then, the light-shielded plant box was prepared; therefore, light could irradiate the aboveground part of the plant in the second generation. In the third generation, the light was off when imaging was performed, so that weak radiation energy could be detected. The third generation enabled us to image the behavior of $^{14}\text{CO}_2$ gas and ^{14}C -photosynthate

Figure 4.30 shows the successive ^{32}P -phosphate uptake images of 3-day-old rice seedlings (*Oryza sativa* cv. Nipponbare) during 60 h of culture in soil and water. One of the rice plants was grown in 22.5 mL of water culture solution (Hoagland medium) containing 1.5 MBq of ^{32}P -phosphate. The other was grown in 20 mL (32 g) of nursery soil for rice seedlings (Kumiai Baido, Kasanen Industry Co, Japan, 3.1 g phosphate per 20 kg), which was mixed well with 15 mL of culture solution containing 1.5 MBq of ^{32}P -phosphate. The integration time for each imaging frame was 3 min.

In the water culture, the rice plant continuously absorbed higher amounts of ^{32}P -phosphate and grew much faster than the plant growing in soil. In contrast, in the soil culture, only a small amount of ^{32}P -phosphate was absorbed from the roots since phosphate was firmly adsorbed in the soil. In the soil culture, it was also observed that hardly any ^{32}P -phosphate was transferred to the aboveground parts, even after 20 h, and the growth of the plant was very slow. Because of the nature of the phosphate ion, which is weakly mobile owing to a very low coefficient of diffusion (10^{-12} to 10^{-15} m^2/s), the uptake of phosphate from the soil created a depleted area around the root. This depletion zone was observed as a dark colored

Fig. 4.30 Comparison of the ^{32}P -phosphate uptake by rice seedlings between water and soil culture [15]. Successive images of ^{32}P -phosphate uptake by the rice seedlings during 60 h of water and soil culture are shown. The integration time for each imaging frame was 3 min. For each record, the sample grown in soil is on the left, and the one grown in the water culture solution is on the right. (a) Picture of the sample. (b) Two ROIs (region of interest) in the ^{32}P image. The blue and red ROIs are the aboveground parts of the rice grown in soil and water, respectively. (c) ^{32}P -phosphate uptake curve in two ROIs. The gray columns in C are the dark period. Pseudocolor was assigned to the image according to the intensity of the radioactivity

area at the root, in the shape of the root, and clearly demonstrated that the phosphate adjacent to the root was taken up by the root. This depletion zone appeared within a few hours, and this area induced further movement of phosphate toward the root, as revealed by the increase in phosphate uptake.

Imaging by the RRIS also offers a way to investigate the effects of light and/or circadian rhythms on phosphate uptake. The light conditions of the plant were a 16 h L/8 h D light/dark cycle during the 60 h of imaging. When the amount of phosphate at the ROI in water culture, as indicated in Fig. 4.30, was plotted successively, the phosphate uptake clearly increased during the daytime. Similar observations have been performed with other plants, such as *Lotus japonicas* (data not shown). Indeed, light conditions or circadian rhythms have been found to directly or indirectly affect ion uptake, and such phenomena could have multiple and complex origins.

It is generally known that when the growth of plants in water culture and soil culture is compared, the growth of the plant grown in water culture is very fast compared to that of a plant grown in soil. This is one of the reasons water culture is employed in factories to grow vegetables; however, it is also known that cereals,

including rice, have much higher yield when grown in soil; therefore, indoor factories are not suitable to grow rice or wheat.

4.3.1.2 ^{137}Cs Absorption in a Rice Plant

After the Fukushima nuclear accident, the movement of ^{137}Cs in soil attracted attention. Although we found that ^{137}Cs was adsorbed firmly on the clay in soil, the absorption of ^{137}Cs is sometimes studied using plants growing in water culture. Therefore, it was necessary to visualize the ^{137}Cs uptake behavior of rice plants growing in soil and to compare it to that of rice plants growing in water culture, partly to show the results to people who are concerned about the contamination of plants grown in contaminated soil.

The rice seedlings with three expanded leaves were grown in 3 mL of liquid medium or soil medium (3 g of soil plus 3 mL of liquid medium) containing 50 kBq of ^{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 the deep part of the paddy field (5–10 cm from the surface). Therefore, in both cases, ^{137}Cs was supplied to both water and soil.

As shown in Fig. 4.31, the rice absorbed a high amount of ^{137}Cs from the root when cultured in water medium, whereas ^{137}Cs was hardly absorbed from the soil, which showed the same phenomenon as that of the ^{32}P supply cited in the previous section. When the radioactivity of liquid and soil was plotted, it was also confirmed that the radioactivity in soil hardly changed with time. The movement of ^{137}Cs uptake in both media was also presented as a movie.

In the case of water culture, the rate of increase in the ^{137}Cs content in leaves declined significantly in several hours, showing that the absorption of ^{137}Cs was completed. The first curve of rapid translocation of ^{137}Cs to the shoot within 5 h could be interpreted as the xylem loading activity, and the subsequent slowly increasing curve that appeared after 5 h may be explained by the ^{137}Cs remobilization activity.

This research was further developed to study ^{137}Cs uptake and xylem loading activities using plants grown under K-deficient conditions. The other findings were that the accumulation of ^{137}Cs within the rice plant during the developmental stage was different from that in a soybean plant, which resulted in a higher ^{137}Cs amount in the edible part of the soybean plant than that of the rice plant (data not shown).

4.3.2 *Multielement Absorption*

4.3.2.1 Multielement Absorption Images in Arabidopsis by RRIS

Since the RRIS system enabled visualization of the element absorption behavior, live imaging of the other nuclides is shown. The first presentation is the visualization

Fig. 4.31 Comparison of the ^{137}Cs uptake by the rice seedlings between water and in soil culture [16]. (a) Rice seedlings were set in the plant box, and real-time images were acquired by RRIS for 20 h. The integration time for each imaging frame was 10 min. The seedlings were grown in water culture until they developed three expanded leaves (L2, L3, and L4) under 16 h L/8 h D, light/dark conditions at 30 °C. Then, two seedlings were grown in 3 mL of liquid medium or soil medium (3 g of soil plus 3 mL of liquid medium) containing 50 kBq ^{137}Cs . The soil was non-contaminated and collected from a paddy field in Fukushima district. In the real-time images, six ROIs were set. 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) IP images of the rice seedlings after the RRIS imaging were completed. The ^{137}Cs signal was hardly detected in the L2 and L3 sheaths. (c) ^{137}Cs accumulation in the six ROIs

of long-distance ion transport in *Arabidopsis* using radioisotope tracers, $^{22}\text{Na}^+$, $^{28}\text{Mg}^{2+}$, ^{32}P -phosphate, ^{35}S -sulfate, $^{42}\text{K}^+$, $^{45}\text{Ca}^{2+}$, $^{54}\text{Mn}^{2+}$, $^{65}\text{Zn}^{2+}$, $^{109}\text{Cd}^{2+}$, and $^{137}\text{Cs}^+$, supplied from the roots. Seeds of *Arabidopsis thaliana* Col-0 were grown in full-nutrient culture solution at 22 °C under 16 h L/8 h D light/dark conditions with 100 $\mu\text{mol}/\text{m}^2$ s of light. After 43 days, plants approximately 25 cm in height were selected and transferred to 20 mL of culture solution containing radioactive tracers of individual nutritional elements. The tracer concentrations applied were as follows: $^{22}\text{Na}^+$, 25 kBq/mL; $^{28}\text{Mg}^{2+}$, 25 kBq/mL; ^{32}P -phosphate, 50 kBq/mL; ^{35}S -sulfate, 500 kBq/mL; $^{42}\text{K}^+$, 1 kBq/mL; $^{45}\text{Ca}^{2+}$, 250 kBq/mL; $^{54}\text{Mn}^{2+}$, 50 kBq/mL; ^{65}Zn , 75 kBq/mL; $^{109}\text{Cd}^{2+}$, 50 kBq/mL; and $^{137}\text{Cs}^+$, 25 kBq/mL. $^{42}\text{K}^+$ was prepared from an ^{42}Ar - ^{42}K generator by milking. ^{28}Mg was produced by the ^{27}Al (α , 3p) ^{28}Mg reaction and separated from the Al target. The imaging area was the aboveground parts between 3 and 22 cm from the root. Samples were irradiated under a light intensity of 100 $\mu\text{mol}/\text{m}^2$ s for 15 min at intervals of 15 min. During each 15-min interval, imaging was performed without light. The radiation converted to photons by the scintillator was harvested for 15 min with a highly sensitive CCD camera (C3077-70, Hamamatsu Photonics Co.).

During 24 h of the radioactive ion supply, the accumulation pattern and uptake speed of each element exhibited specific features. From Figs. 4.32, 4.33, and 4.34, each element uptake image with time is shown, and pseudocolor was added according to the intensity of the radioactivity. When these successive figures in each element were connected, a movie was produced to show the movement.

Sequential analysis showed three distribution patterns in the aboveground part of *Arabidopsis*. The first was a widespread distribution over time, as exhibited by ^{22}Na , ^{32}P , ^{35}S , ^{42}K , and ^{137}Cs . The second pattern shown by elements ^{28}Mg , ^{45}Ca , and ^{54}Mn was a higher accumulation in the basal part of the main internode. The third pattern was that accumulation was only found in the leaf tips or the bottom parts of the flower, as in the case of ^{65}Zn or ^{109}Cd . In the case of ^{45}Ca , ^{28}Mg , or ^{54}Mn , only a small amount of the ion reached the tip of the stem, even after 24 h, and the movement was very slow. In contrast, the heavy elements ^{65}Zn and ^{109}Cd moved very fast, and when they were transferred to the aboveground part, they suddenly moved to the leaves without accumulating in other tissues and accumulated at the leaf tips. Representative examples of the three element profile patterns after 24 h are shown in Fig. 4.35.

The difference in the absorption images, indicating the differences in distribution and speed of movement, seemed to be derived, at least in part, from the chemical forms of the elements; one group comprised monovalent cations or anions such as ^{22}Na , ^{42}K , and ^{137}Cs , whereas the other group was multivalent cations, such as ^{28}Mg , ^{45}Ca , and ^{54}Mn . Given the widespread distribution profile along the main stem from the lower to the upper parts, monovalent cations and anions appeared to move through the vascular tissue smoothly and quickly, whereas multivalent cations moved slowly. The low velocity of multivalent cation transport is possibly derived from the interaction between the ions and the negatively charged cell wall of xylem vessels.

Fig. 4.32 Successive images of the ion movement in Arabidopsis taken by RRIS (1) [17]. ^{22}N (a), ^{28}Mg (b), and ^{32}P (c) were supplied to the roots. The detection time for each imaging was set to 15 min

4.3.2.2 Mg Movement in Arabidopsis

Among the elements investigated, the characteristic transport of ^{28}Mg within the main stem of the inflorescence is presented as an example. When two regions of interest (ROIs) were set at different parts of the internode, the difference in radioactivity counts with time shows the movement of the element more clearly. Two ROIs were set at an interval of 30 mm (Fig. 4.36). The signal intensity of ^{28}Mg in the ROI: A, which was set at the lower position, exceeded the limit of quantitation (LOQ) soon after the imaging was started and continued to increase linearly. The LOQ corresponds to the earliest time when the radioisotope was first able to be detected. Subsequently, after approximately 6 h, the ^{28}Mg content in ROI: B, which was set at the higher position, began to increase linearly. According to the time gap between ROI:A and ROI:B, the time required for ^{28}Mg to travel 30 mm was 5.5 h. Accordingly, the velocity of Mg^{2+} toward the top of the main stem was estimated to be 5.5 mm/h. By similar measurement, the velocity of ^{32}P was calculated to be >60 mm/h.

Fig. 4.33 Successive images of the ion movement in Arabidopsis taken by RRIS (2) [17]. ^{35}S (d), ^{45}Ca (e), and ^{54}Mn (b) were supplied to the roots

Fig. 4.34 Successive images of the ion movement in Arabidopsis taken by RRIS (3) [17]. ^{65}Zn (h); ^{109}Cd (i); ^{137}Cs (j)

The amount of ^{28}Mg delivered to the upper part of the plant was small. However, Mg is required at the tip of the main stem of the inflorescence. Together with limited information about the movement of Mg, the accumulation behavior at each node was

Fig. 4.35 Representative three patterns of the element profile after 24 h. ^{32}P group: widespread distribution throughout the plant; ^{28}Mg group: very slow movement, accumulated at the basal part of the main internode; ^{109}Cd group: very fast movement, accumulated at the leaf tips and bottom parts of the flower

Fig. 4.36 (d) Time-course analysis of the radioactivity of ^{28}Mg detected in two ROIs [17]. ROI A: a blue circle was set on the main stem, 30 mm upper part from the top node. ROI B: a red circle was placed 30 mm above ROI A. The solid line shows the limit of quantitation (LOQ). The broken line shows the limit of detection. The linear components in the upper graph were extracted and are shown in the lower graph

Fig. 4.37 Analytical method of the transfer/accumulation ratio of ^{28}Mg at the nodes [3]. ROIs A1–A4: each 1.5 mm in length, and the number increased with height. A1 was set at the internode, and A2–A4 were set at the nodes. ROIs B1–B4: each ROI B is the upper part of the corresponding ROI A area. *vb* vascular bundle

analyzed [3]. To estimate whether Mg accumulated at this part for transfer to the connected branch or was delivered only to the upper main stem, the ratio of Mg accumulated at a certain part of the node to all the Mg absorbed above this node was calculated. As shown in Fig. 4.37, one internode and three nodes above this part, each 1.5 cm in length, were selected (A1–A4), and the part of the plant higher than each position of A was selected (B1–B4). At all sites, A1 to A4 and B1 to B4, the absorption curve linearly increased (Fig. 4.38). Then, the accumulated ratio at each A position was calculated as $A/(A + B)$. From this ratio, the loading manner of Mg from xylem tissue could be estimated, namely, when the ratio is high, the accumulation character as a sink is high and the low ratio indicates the low accumulation amount at the site and Mg was just transferred to the upper part of the plant. When the ratio was calculated, there was no change in the ratio of the accumulation at each node with time, and the accumulation rate increased as the position of the node became higher (Fig. 4.39). The results indicated that the ratio of Mg transfer from xylem tissue at the node was kept constant, and the accumulation rate was higher at the upper part of the stem, suggesting that the speed of the transfer movement of ^{28}Mg decreased as the height of the internode increased.

However, after the ions reach the bottom part of the shoot, the part played by the phloem in promoting ion transport should be considered in addition to xylem flow. To evaluate phloem contribution to ion transport, heat girdling was performed for

Fig. 4.38 ^{28}Mg intensity with time at ROIs A-B in Fig. 4.37 [3]. (a) ^{28}Mg intensity in ROIs A1-A4; (b) ^{28}Mg intensity in ROIs B1-A4

Fig. 4.39 Accumulation ratio of ^{28}Mg at ROIs An in Fig. 4.37 [3]. (a) accumulation ratio at the internode (A1) and nodes (A2–A4); (b) accumulation ratio of A1-A4 with height

Arabidopsis grown for the same period as used in the previous section, 43-day-old seedlings. The main stem was heated for several seconds by a soldering iron. First, 1 MBq of $^{14}\text{CO}_2$ was supplied to the rosette leaves, and the image of the whole plant was taken by IP to confirm the reliability of the heat-girdling technique. Since the IP showed no photosynthate image before or after the treatment in the internode of the plant (data not shown), 10 kBq/mL of ^{28}Mg was supplied to the plant, and the absorption curve was obtained from the successive images acquired by RRIS. The ^{28}Mg distribution pattern along the main stem was similar to that of nontreated plants (Fig. 4.40). A kinetic analysis showed that the velocity of Mg^{2+} in the xylem flow was 5.5 mm/h, a similar value to that found in intact Arabidopsis cited above. Thus, the upward Mg^{2+} movement within the third internode of the main stem is likely to be mediated mainly by xylem flow, while the phloem contribution is scarce during the first 24 h of root absorption. In contrast, in the case of ^{32}P -phosphate absorption,

Fig. 4.40 ^{28}Mg uptake image of Arabidopsis after the heat-girdling treatment [17]. Heat-girdling treatment was performed at the position marked with a red arrow (upper left). The exposure time of the camera was set to 15 min. The ROIs are indicated by blue circles (ROIs A) and red circles (ROIs B) in the image. Upper: distribution images of ^{28}Mg within 24 h after the treatment. Lower: signal intensity of ^{28}Mg in ROI A and ROI B. The linear components were extracted (lower right)

heat girdling resulted in strong ^{32}P signal accumulation at the bottom of the main stem, which was never observed in untreated Arabidopsis (data not shown).

4.3.2.3 Mg and K Absorption in a Rice Plant

Rice plant seedlings after 12 to 14 days of germination were used to obtain uptake images of ^{28}Mg , ^{32}P , ^{35}S , ^{42}K , and ^{45}Ca for 12 h. ^{28}Mg and ^{42}K images are shown in Fig. 4.41.

The ROI (region of interest) was set at the third and fourth leaves, and the uptake of both ^{28}Mg and ^{42}K was plotted. It was shown that the accumulation patterns of both elements are different. The amount of Mg in both leaves was similar, and the amount increased linearly until 12 h. With further development of this study, applying this ^{28}Mg imaging technique, an early response of Mg deficiency was found to appear, especially in the fifth leaf [18]. This result was further examined to determine the mechanism of Mg absorption and translocation, using ^{28}Mg as a tracer (data not shown). In the case of K, there was a drastic difference in the accumulation amount between the third and fourth leaves. The absorption speed and the amount of K in the third leaf were more than two times higher than those in the fourth leaf,

Fig. 4.41 ^{42}K and ^{28}Mg uptake images in rice plants [2] modified. The ROIs were set at L3 (third leaf) and L4 (fourth leaf). L4 grew after L3, and the signals at these ROIs were plotted

suggesting a quick K movement response, especially to tissue where the K requirement was high. Since K showed a competitive character for Cs absorption, application of K was found to inhibit ^{137}Cs absorption from the root when ^{42}K was applied as a tracer. ^{42}K was further used to study the mechanism of ^{137}Cs contamination caused by the Fukushima nuclear accident.

4.3.3 Summary of Element Absorption from Roots

Since radiation can penetrate both water and soil, it was revealed that there was a great difference between water culture and soil culture in growth as well as ion absorption from roots. It is rather popular to perform physiological studies of plants employing water culture; however, extending the results to understand plants growing in the field reveals discrepancies in physiology between plants grown in water and those grown in soil, i.e., the plant physiology is totally different.

For example, as cited above, it is generally known that the grain yield of rice grown in soil is much higher than that of rice grown in water culture. Although it is not known what determines the yield of the plant, the fact that slowly growing plants produce higher amounts of seeds might suggest that the different usage of energy by the plant during growth, such as the higher energy requirement for the absorption of phosphate from soil, might affect the production of many grains. The roots actually use large amounts of energy to remove phosphate from the closest soil to absorb. As a result, there was always ^{32}P -depleted zone whose shape reflected that of the root itself. Soil culture is very complicated because various physiological and biological factors are in the soil itself; however, for soil culture, the application of RI is an indispensable tool to study the physiological aspects of plants.

The real-time RI imaging system (RRIS) was applied to visualize multielement absorption in a plant. The absorption velocity is very different among the elements, which results in different distribution patterns within a plant. In the case of

Arabidopsis, the element-specific absorption patterns were clearly classified into three patterns within 24 h of root absorption. It was very interesting to note that so many ions with such different velocities are actually moving in water, which is also flowing upward from the root in the xylem.

Since the image was produced based on radiation, image analysis could be performed, and the case of ^{28}Mg is presented as an example. Through image analysis, it was possible to differentiate xylem flow from phloem flow. In the case of ^{28}Mg , most of the flow was through the xylem, and phloem flow was hardly observable within 24 h of transport. Applying this imaging method, the specific element accumulation sites were also detected.

4.4 Development of a Microscopic Real-Time RI Imaging System (RRIS)

The RRIS presented above was for a relatively large-scale sample, for example, a whole plant or whole tissue. However, to perform microscopic RI imaging, different types of solutions must be developed to enable the desired level of magnification. Therefore, a new system microscope-modified system was developed, integrating a thin Cs(Tl)I scintillation system and a magnification device.

4.4.1 *Modification of a Fluorescence Microscope*

The first step was the preparation of a scintillator. The scintillator thickness determines the resolution and sensitivity of the radiation image, where a thinner scintillator provides higher resolution; however, higher sensitivity is acquired with increasing thickness. The penetration of radiation through the scintillator is another factor to take into account, especially when the β -ray energy is high. Therefore, to install the scintillator for the microscope, the thickness of the scintillator (Cs(Tl)I) should be properly prepared with respect to the sensitivity and resolution. Several kinds of Cs(Tl)I scintillators were prepared with different thicknesses of Cs(Tl)I, 10, 25, 50, 100, and 200 μm , deposited on a fiber optic plate (FOS) in vacuum. The standard samples were 0.37 kBq of ^{32}P , 1.85 and 3.7 kBq of ^{45}Ca and 0.925 and 1.85 kBq of ^{14}C , prepared from $\text{H}_3^{32}\text{PO}_4$, $^{45}\text{CaCl}_2$, and ^{14}C -glucose solutions, respectively, and mounted on a membrane filter. The filter mounted with the spots was covered with a Mylar film (4 μm). Then, an FOS was placed on the filter with the standards, and the measurement was performed for 3 min by a GaAsP imaging intensifier unit with a detection area of $5 \times 5 \text{ cm}$ (See 4.2.2 of this chapter).

To address resolution, a membrane filter soaked with ^{32}P solution (3.7 kBq/ μL) was prepared. On this filter, two iron plates 1 mm in thickness were placed parallel to each other at a distance of 500 μm . Then, FOSs with different scintillator thicknesses

Fig. 4.42 Thickness of the Cs(Tl)I scintillator of the fiber optic plate (FOS) and detection efficiency [19]. Cs(Tl)I scintillators with different thicknesses were prepared by depositing on FOS in a vacuum. The standard solutions of ^{32}P , ^{45}Ca , and ^{14}C were prepared as spots on a membrane filter

were placed on the sample, and the β -ray image of the slit was analyzed. The detection limit was set to twice as high as the background intensity.

Figure 4.42 shows the relation between the thickness of the scintillator, Cs(Tl)I, and the detection efficiency. As shown in the figure, the detection efficiency depends on the energy of the radiation from the nuclide, where ^{32}P (β -ray energy max: 1.709 MeV) continuously increased with increasing thickness from 10 to 200 μm . In the case of ^{14}C (β -ray max: 0.156 MeV) and ^{45}Ca (β -ray max: 0.257 MeV), the detection efficiency plateaued after 50 and 100 μm , respectively. The resolution of the image increased with decreasing scintillator thickness (Fig. 4.43). A 50 μm thickness of the scintillator deposition was selected as preferable for imaging a nuclide with lower β -ray energy, such as ^{45}Ca or ^{14}C .

Once the thickness of the scintillator was decided, the next step was the magnification method. In the microscopic imaging system, a taper FOS was applied (Fig. 4.44), which allowed five times magnification. The diameter of the FOS was 3 μm on one side and 15 μm on the other side. The scintillator was deposited on the

Fig. 4.43 Thickness of the Cs(Tl)I scintillator and image resolution [19]. Two parallel iron plates that formed 500- μm slits were placed on the RI sheet, and FOSs with different scintillator thicknesses were placed on the slit. The line profile of the slit image produced by the FOS was analyzed to obtain the resolution

surface of the smaller end. The surface of the tapered FOS was covered with an aluminum Mylar to prevent contamination as well as background light. As shown in the figure, the system consisted of the FOS, a lens, and a GaAsP imaging intensifier. To obtain higher-resolution images, an Axio Cam HRm (Carl Zeiss, Co.) was applied to acquire fluorescent images in the imaging intensifier unit, and Axio Vision (Carl Zeiss, Co.) was used for image analysis.

The transmitting light unit was removed, and a new pole was installed for the microscope so that the real-time imaging system could be smoothly shifted vertically by an electric motor. After bright field and fluorescence images were taken, the scintillator side of the tapered FOS was placed on the sample, and the radiation image was taken from 3 to 10 min of integration.

Fig. 4.44 Schematic illustration of micro-RRIS [19]. A fluorescence microscope was revised to acquire radiation images and fluorescence images. Taper FOS was prepared, which enabled five times magnification of the image, and the scintillator was deposited on the smaller end area. To acquire radiation images, a metal tube consisting of the taper FOS, lens, and GaAsP imaging intensifier was installed

4.4.2 Radiation Images Under the Modified Fluorescence Microscope

With the modification of the transmitting light unit, the fluorescence microscope could acquire three kinds of images: light image (BF camera), fluorescence image (FL camera), and radiation image (RI camera). Figure 4.45 shows a picture of the modified fluorescence microscope and an example of the three types of images acquired from the same soybean stem dissection sample when ^{45}Ca was supplied. The resolution of the ^{45}Ca image was not very high, since the thickness of the sample was 70 μm , which was the estimated size of a single cell. With this thickness, the β -ray irradiated from the sample was somewhat expanded before reaching the scintillator, resulting in an image with low resolution.

Under this modified fluorescence microscope, the distribution of different radioisotopes (^{45}Ca , ^{35}S , and ^{55}Fe) in various tissues of *Arabidopsis* was observed, and the radioisotope images with pseudocolors were superimposed on the corresponding light images (Fig. 4.46). With the $^{45}\text{CaSO}_4$ solution, images were obtained 1 h after supplying the solution to roots. A higher accumulation of ^{45}Ca was observed in younger leaves. On the other hand, in the case of ^{35}S , it took hours before ^{35}S was detected in leaves. The image of ^{35}S distribution in Fig. 4.46 was obtained after 48 h of $\text{Na}_2^{35}\text{SO}_4$ supply, and ^{35}S was observed to accumulate along the leaf veins.

As another example, Fig. 4.46 shows successive images of ^{55}F distribution acquired every 20 min after $^{55}\text{FeCl}_2$ solution (100 KBq/25 μL) was supplied to a 3-day-old *Arabidopsis* seedling. The integration time of each imaging frame was 2 m. The figure illustrates the accumulation of ^{55}F in the root tip.

Fig. 4.45 Micro-RRIS with 3 types of images, radiation image, fluorescent image, and light image. ^{45}Ca solution (10 M Bq/20 mL, Ca: 20 μM) was supplied to the stalk of the first trifoliate leaves of a soybean plant after 3 weeks of germination. Then, the stalk was sliced to 70 μm , and a radiation image of ^{45}Ca was obtained. The neighboring slice was stained with fluorescent dye Fluo-3 M to acquire a fluorescent image of Ca

4.4.3 Further Modification of Micro-RRIS

Then, further improvement of the micro-imaging system was performed. To achieve a higher magnification, an optical lens was introduced instead of a tapered FOS. A combination of the FOS and an optical lens magnified the image 20 or 40 times, depending on the lens used (Fig. 4.47).

Since the image is based on radiation, quantitative analysis can be performed. To confirm the quantitative character of the image, the radioactivities calculated from the images of standard solution spots of ^{32}P and ^{35}S were compared with the actual radioactivities of the standard solutions prepared, ^{14}C , ^{55}Fe , ^{32}P , ^{35}S , and ^{109}Cd . In all cases, the linearity of the signal was conserved between the counts of the image (cpm) and the radioactivity of the mounted standard solution, which facilitated imaging over a broad range of concentrations. Out of 5 nuclides, Fig. 4.48 provides the results of ^{32}P and ^{35}S . As shown in the figure, in both cases, the linearity of the signal was conserved between the counts of the image (cpm) and the radioactivity of the standard solution prepared (Bq). Conservation of the linearity ranged from 0 to 6.5 kBq/ μL (^{32}P) or even to 28 kBq/ μL (^{35}S). In all cases, such signal dynamics far exceed the signals used in experiments. It was shown that this micro-imaging system was thus applicable to a broad range of experiments. For example, in the case of ^{32}P solution, it was possible to detect the image of a 1 μL spot containing only 16 Bq in 2 min.

Fig. 4.46 Example of the images acquired by micro-RRIS in Arabidopsis [15]. For ^{45}Ca imaging, $^{45}\text{CaSO}_4$ solution (1 MBq/0.5 mL) was supplied to the root of a 13-day-old seedling for 1 h. The $\text{Na}_2^{35}\text{SO}_4$ solution (5 MBq/0.5 mL) was supplied to the root for 48 h. In both plants, imaging was performed after 10 h. To obtain successive images of the iron uptake, $^{55}\text{FeCl}_2$ solution (100 kBq/25 mL) was supplied to a 3-day-old seedling; ^{55}Fe accumulation was observed in the root tip

To confirm the linear relation between the radioactive counting in the image of the plant and the actual radioactivity of the tissue measured by liquid scintillator, an Arabidopsis plant was employed. The plant was grown for 10 days in 1/10 MS medium without phosphate, after which ^{33}P -phosphate solution (6 kBq/μL) was supplied for 5 min, and the root was imaged for 5 min. After imaging, the root tip (2 mm) was digested, and the radioactivity of ^{33}P was measured by a liquid scintillation counter. Then, the amount of ^{33}P measured in the image of the root grown under phosphate-deficient conditions was compared to that of a root grown under sufficient conditions. When ^{33}P -phosphate was supplied to the culture solution, the amount of ^{33}P accumulated in the root tip grown under phosphate-deficient conditions was 7–8 times higher than that grown under normal conditions, which

Fig. 4.47 Modification of micro-RRIS [15]. To achieve a higher magnification, a combination of an FOS without taper and an optical lens was prepared, which magnified the image 20 or 40 times depending on the lens instead of using a taper FOS

was in good accordance with the actual data on harvested roots measured by a counter (Fig. 4.48).

Then, what kind of magnified root image could be shown under this further modified microscope? Figure 4.49 shows an example of a ^{32}P image of a 10-day-old seedling of *Arabidopsis* root when 100 Bq/ μL of ^{32}P -phosphate was supplied for 5 min. The plant was placed on a glass slide, and a radiation image was taken. As shown earlier, phosphate was shown to accumulate in the root tip. When the root tip marked in the square was magnified and the light image was superposed with the ^{32}P image, the phosphate distribution in the root tip was visualized. In the past, an imaging plate (IP) was used to analyze phosphate uptake locations, and the root tips were identified as an important area for phosphate uptake. Nevertheless, the resolutions in the previous experiments were far from those obtained here, where it was possible to clearly visualize the labeling of the meristem area, distinct from the uptake at the level of root hairs above the area of differentiation.

Figure 4.50 compares the root site where the phosphate transporter was expressed and the position where ^{32}P -phosphate was actually taken up. As clearly shown in the figure, phosphate was actually taken up from the site where the transporter gene was expressed. This imaging study was further developed to illustrate the importance of the role of the PHT1 family of phosphate transporters [20].

Finally, the resolution of the micro-RRIS is described. To investigate the resolution of the image, for example, a standard grid sample prepared by metals is commercially available for electron microscope imaging. However, standard grid samples prepared with radioisotopes (RIs) are not available. Therefore, the RI grid had to be prepared in our laboratory to estimate the resolution of the micro-images. The RI grid was prepared by printing RI lines with a printer where ^{137}Cs was mixed

Fig. 4.48 Quantitative analysis of the image acquired by micro-RRIS [15]. **(a)** Arabidopsis was grown in sufficient or deficient conditions of phosphate for 10 days, ^{33}P -phosphate solution (6 kBq/ μL) was supplied for 5 min, and the root was imaged for 5 min. The radioisotope image of the root superposed on the light image showed a higher ^{33}P signal at the root tip when grown under phosphate-deficient conditions. **(b)** Measurements of plant radioactivity in the root tip (2 mm). The gray and white columns show the radioactivity counts of the root tip measured by a scintillation counter and image analysis, respectively. **(c)** Calibration curves of the counts of the image versus the radioactivity of the standard solution

with ink. The width of the line was 50 μm , and the distance between the lines was 450 μm (Fig. 4.51). From the image, the resolution of the micro-imaging system was estimated to be approximately 50 μm .

Fig. 4.49 ^{32}P uptake image of Arabidopsis root with higher magnitude [15] modified. ^{32}P -phosphate ($100 \text{ Bq}/\mu\text{L}$) was applied to 10-day-old seedlings for 5 min, and the roots were imaged for 3 h. The isotope image was superposed on the corresponding light image, and pseudocolor was added according to the intensity of radioactivity. Using a modified micro-RRIS, a ^{32}P image was acquired at higher magnification with an optical lens (20 times)

4.5 Summary and Further Discussion

Real-time radioisotope (RI) imaging systems were developed step by step to image nutrient uptake in plants using not only conventional β -ray, γ -ray, or X-ray emitters but also additional produced radioisotopes, such as ^{28}Mg or ^{42}K . These methods provide specific and direct imaging possibilities for many ions where no alternative solution with fluorescent probes exists. The image allowed quantitative analysis to calculate the amount of the element and offered a wide dynamic range of detection. Therefore, it was possible to study the ion influx from culture medium over time periods as short as 1 or 2 min or over a period of a few days. This method also enabled us to conduct several types of pulse chase experiments, which cannot be performed using other tools, such as fluorescence techniques, which are mostly restricted to distribution analyses. The RIs we could test included over 10 nuclides in total. When the radiation energy is lower, the specific activity of the RIs should be higher; that is, a higher dose is necessary to acquire the image.

Through real-time imaging of the radioisotope movement, ion-specific speeds or accumulation patterns throughout the whole plant can be acquired. Among the elements investigated, there were three types of movement patterns in Arabidopsis.

Fig. 4.50 ^{32}P image of Arabidopsis root, control, and mutant. (a) control sample; (b) mutant in which the phosphate transporter gene is expressed only at the root tip. *Upper:* ^{32}P images; *Lower:* gene expression images. There was a correlation between the gene expression area and the ^{32}P uptake area. Bar: 100 μm

These ions are dissolved in water and therefore seem to move in the water but not with water. When the movement of water was measured, as described in Chap. 2, it showed constant speed and a constant route and expansion of the movement. On the other hand, the ion movement here showed specific speeds unrelated to the water movement itself.

This means that each element produces a specific concentration gradient from tissue to tissue. These profiles or movement might be different with time and change with the developmental stage. Thus, plants show very sophisticated and complicated element movement and profiles, and we do not have any means to understand the balance among all these movements.

Another interesting issue is how plants are absorbing elements. As was written regarding neutron imaging, many questions of root activity were raised when the movement of water or ions toward roots or within the plant was visualized. In this

Fig. 4.51 Grid image of ^{32}P under micro-RRIS. To estimate the resolution of the image, ^{32}P was mixed with ink of the printer, and the grid pattern was drawn. The printed ^{32}P line width was $50\mu\text{m}$ at an interval of $500\mu\text{m}$. Both bright field image (*left*) and ^{32}P image clearly reveal the printed lines

section, it was possible to show the difference in root activity in absorbing phosphate between water culture and soil culture.

Two types of real-time RI imaging systems (RRIS) have been presented, one for macroscopic imaging and the other for microscopic imaging of the plants treated with RIs. One of the purposes of macroscopic imaging is to pursue the possibility of tracing RIs without any influence on growth conditions, including illumination, and to image a wide range of the elements that contribute to plant growth. In the case of microscopic imaging systems, further development of the devices is needed. For example, developing a small growth chamber for plants with nutrient circulation and an irradiation system is now another goal. The resolution of microscopic imaging was approximately $50\text{--}100\mu\text{m}$.

It should be noted that additional equipment mounted on the microscope is needed to proceed with the acquisition of light signals, fluorescence or luminescence, at the same time, which could be combined or superposed with radioactive imaging. This system offers a broader range of applications, such as measuring the effects of the genetic manipulation of transporters labeled with green fluorescent protein or luciferase on ion transport in specific plant tissues.

The development of these macroscopic and microscopic imaging systems will facilitate the systematic analysis of the real-time uptake of various macro- and micronutrients, from the macroscopic level to the microscopic level, i.e., from the whole plant to the cellular level. Because these visualizations allow numerical analysis of the image, we expect that isotope images will open a new avenue in plant physiology.

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