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A study of ^{32}P-phosphate uptake in a plant by a real-time RI imaging system

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Summary. It is very important to visualize the process of nutrient absorption and distribution to study the physiological activity of the plant. We developed a real-time radioisotope (RI) imaging system, where RI tracers were applied to the plant sample. This system allowed the quantitative measurement concerning the uptake of nutrients labeled with radioisotopes, such as ^{45}Ca, ^{35}S, ^{32}P and ^{14}C as long as several days. The β-rays emitted from the sample were converted to light by a CsI(Tl) scintillator and were guided to a highly sensitive CCD camera. The scintillator surface was covered with an Al plate to avoid LED light penetration but allow selected β-ray penetration. We employed *Lotus japonicus* for the plant sample and observed the ^{32}P-phosphate absorption in roots and the accumulation to the aboveground part of the plant. The environment condition of daytime and night was simulated by the ON/OFF of LED timer and the accumulation manner of the ^{32}P-phosphate in roots and leaves during daytime and night was analyzed. The accumulation of ^{32}P-phosphate in leaves was highly dependant on light irradiation and higher when the LEDs was turned on, whereas the absorption of ^{32}P-phosphate in root was higher when the LEDs was turned off. The transfer function concerning the transportation of phosphate within the plant during the developmental stage was obtained from the analysis of ^{32}P uptake images. We are now trying to get specific moving images of each radioisotope when two kinds of isotopes, such as ^{32}P and ^{35}S, were applied at the same time to the plant, through an image analysis.

1. Introduction

Seventeen elements, including P, Mg, Ca, C, and N, are essential nutritional elements for plant growth. It is of critical importance to analyze how these elements are absorbed from the roots as nutrients, and then distributed to the leaves [1]. In particular, in the case of P, a large quantity of P is contained in DNA, cell membranes and ATP, and it is preferentially distributed to growth tissues, thus gathering a special attention [2]. Though P is a major essential element for plant growth, Japan relies on imports from foreign. With recent shortage of P from the mines, fertilizer prices have increased 1.5 to 2 times in the last few years [3]. Therefore, it is very important to utilize P effectively, especially based on the study to examine the absorption kinetics of P in plants for efficient growth. We developed a system that allowed 2-dimensional real-time observations of a plant sample to analyze absorption, distribution, and storage of various nutrients during its growth. Using this device, it has become possible to obtain real-time radioisotope (RI) video images of ions and chemical compounds labeled with ^{32}P, ^{45}Ca, ^{14}C, and ^{35}S, β-ray-emitting radio nuclides. These images showed processes of those absorption from roots and distribution throughout the plant, over a relatively long period of one week.

The plant was kept under active condition and quantitative analysis of the ion movement was performed based on the real-time images. In our imaging system, the β-rays emitted from the RI in a plant sample was converted to light *via* a scintillator. The converted light was amplified by a Micro Channel Plate (MCP), and then the RI distribution image was obtained using CCD camera. The absorption of nutrients by a plant for growth seems highly dependent on the environmental condition. However, the prototype system could not observe a plant under photo-irradiated condition because the imaging method required complete darkness to detect imperceptibly weak scintillation light. We developed the system to irradiate light onto a plant preparing a sample container that shields light. At the same time, since a scintillator permeates light, we covered the scintillator with 50 μm thick aluminum plate to prevent the light from the light source (LED) to enter into the CCD camera. These measures allowed us to observe a plant under a light condition [4]. Using experimental data obtained from this system, the mechanism for nourishment transmission in the aboveground parts of plant was discussed elementarily [5]. Also, the successive RI imaging of 6 d was achieved keeping the plant in good health under light/dark cycles [6]. Real-time visualization of the uptake and transportation of a certain kind of substance in a plant was successfully performed using positron emitting tracer imaging system (PETIS) and the dynamics of the substances in a living plant and the damage and recovery functions of plants were discussed [7, 8]. Also, there is an example that used a transfer function analysis method for the analysis of the two-dimensional image data obtained with PETIS [9].

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In this research, we employed *Lotus japonicus* as a plant sample. We observed how phosphoric acid labeled with ^{32}P was absorbed into the sample. It was shown that the absorption rates of P were different among the plant tissues during day and night. In this report, the effect of lighting conditions on phosphoric acid absorption and accumulation is discussed. A mathematical model of P translocation within the plant is presented and experimental results were discussed using the model.

2. Materials and methods

2.1 Experimental system

Fig. 1 shows the schematic illustration of the system developed. The roots of the sample plant are submerged in Hoagland's culture medium containing ^{32}P. The ^{32}P in the liquid culture medium was absorbed from the roots and then distributed throughout the plant. The β-ray emitted from the sample was converted into visible light *via* the CsI(TI) scintillator, 100 μm in thickness, which was deposited on the fiber optic plate (FOP). Since the plant was placed close to the scintillator, the distribution image of the ^{32}P was projected onto the corresponding surface of the FOP. Then the image was recorded by a CCD camera after amplification *via* the image intensifier unit (I.I.Unit, Hamamatsu Photonics, Co.). The I.I.Unit is a light-amplifying device consisted of photoelectric surface, MCP, phosphor screen and FOP, capturing weak scintillation light by a CCD camera. Since any weak light becomes noise in capturing the scintillation light, the actual image recording was performed in the dark.

The container for preparing the sample plant was shown in Fig. 2. The container was equipped with 100 LEDs, capable of emitting 9000 lx onto the sample plant. Through complete covering of the plant and the light source, the container prevented the light to produce a noise for the image recording. Since the scintillator allowed light to permeate, the surface of the scintillator facing the container was covered with an aluminum plate 50 μm in thickness. In this way, light emitted from the LEDs was completely shielded and no noticeable deterioration of the β-ray penetrating the aluminum plate and reaching the scintillator was found. The size of the scintillator deposited on the FOP was 10 cm × 20 cm. The LEDs were switched on and off to produce day and night condition, 16 h light/8 h dark. The images were generated by CCD data accumulated for 3 min per each photo frame and were continuously recorded for 60 h.

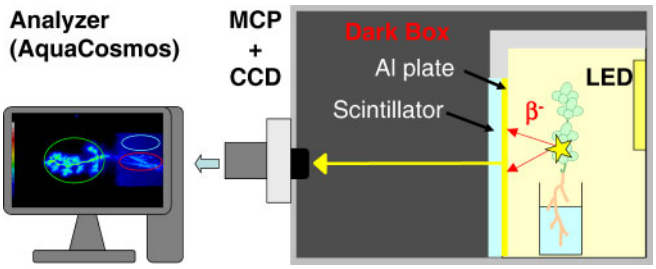

Fig. 1. An imaging system constructed.

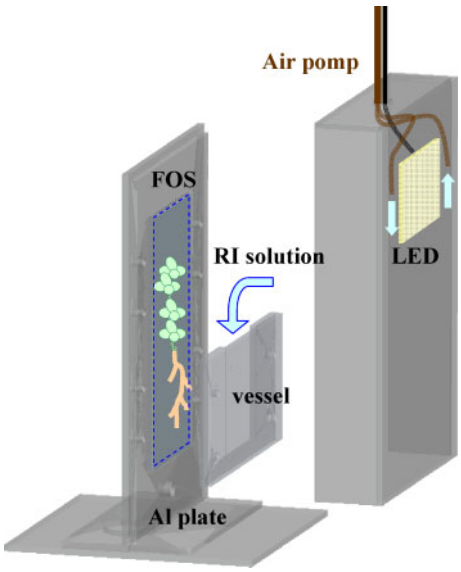

Fig. 2. Assembly of a plant sample container. The root of the sample plant was set in a culture vessel containing ^{32}P. The vessel covered with light shield was placed as close as possible to the surface of FOS.

2.2 Sample and setting

We used *Lotus japonicus* as a plant sample for this experiment. *Lotus japonicus* is widely used as a model plant and the size of the plant is relatively small when grown up with flowers and pods, which is suitable for our imaging system. Fig. 3 shows a sample preparation. The plant that passed about one month after its germination was applied as a sam-

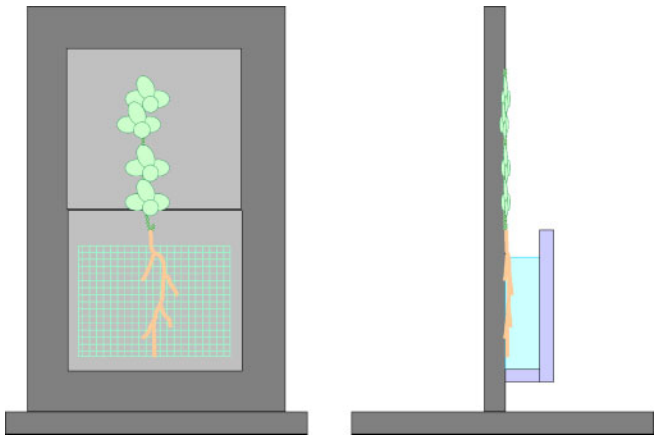

Fig. 3. Sample preparation.

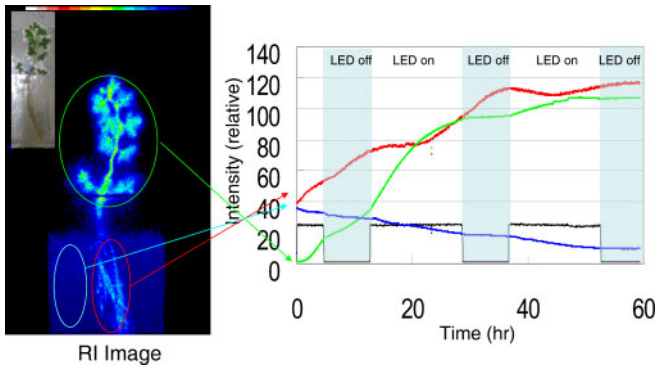

Fig. 4. An image of ^{32}P distribution in plant and relative uptake manner of ^{32}P in each part of the sample.

ple and the size was approximately 15 cm in length. For this experiment, the roots of the sample plant were submerged in the 37.5 ml of Hoagland's medium (P: 1 mM, ^{32}P: 1 MBq/37.5 ml). An Image of ^{32}P distribution in the sample as well as ^{32}P uptake manner is shown in Fig. 4.

3. Results

The left figure in Fig. 4 showed an image of ^{32}P distribution, 30 h after the start of the experiment, where phosphoric acid was distributed throughout the plant. The graph in Fig. 4 showed ^{32}P activity change in culture solution, roots, and aboveground parts of the plant with time. As is shown in the figure, the accumulation process of the phosphoric acid is different in each region. The decrease of radioactivity in the culture solution was relatively linear, indicating a constant rate of P absorption by the plant. However, in aboveground parts of the plant, accumulation of P was higher when the LEDs are turned on compared to that when they are off. On the contrary, the absorption of P by the roots was lower when the LEDs are turned on, and the level of P was even reduced on occasions when lights were on. The absorption manner of the roots related to the lighting condition was opposite to that of aboveground parts of the plant. The experimental results indicate that the accumulation of the phosphoric acid in the plant roots and aboveground parts reached plateau approximately 60 h after the start of the experiment. As mentioned previously, the rate of accumulation of the phosphoric acid to the roots when LEDs are turn on did not remarkably increase like that to the aboveground parts, and rather partially decreased when LEDs are turn on. The rate of transport of the phosphoric acid from the roots to the aboveground part of the plant increased when the LEDs are turned on, thus accumulating phosphoric acid more rapidly into the aboveground. Consequently, this decreased the accumulation of the phosphoric acid into the roots of the plant.

4. Discussions

As is shown to Fig. 4, the aboveground parts of the plant accumulated considerable amount of phosphoric acid while the accumulation in the roots decreased when the LEDs are turned on. It was clear that the phenomenon was not due to the decrease of ^{32}P activity, which half-life is 14 d. As mentioned previously, the cause of this phenomenon may be the rapid transfer of the phosphoric acid from the roots to the aboveground. When LED light was on, there was a high accumulation of P at aboveground parts but low accumulation in roots. On the other hand, the increased accumulation of the phosphoric acid in the roots while LED light was turned off might be attributed to the regulated phosphoric acid transfer from roots to aboveground parts of the plant. Let us discuss whether we can understand this phenomenon based on the transportation of the l phosphoric acid within the plant.

Suppose that there are fixed volumetric capacities in the roots, stems, and leaves of the plant and in the culture solution itself for receiving culture solution containing phosphoric acid. We term the capacities of three areas, where we observe RI within partial zone S_A of culture solution, S_B of roots, and S_C of aboveground parts, as A, B, and C, respectively. These volumetric capacities were regarded as the volume of the plant tissues observed where culture solution flowed. If we define the radioactivity count of ^{32}P within capacities, A, B, and C, as $p(t)$, $m(t)$, and $n(t)$, and volumes of the solvent transferring from S_A to S_B, S_B to S_C, and S_C to outside of S_C in elapsed time t, as u, v, and w respectively, we can write differential equations for $p(t)$, $m(t)$, and $n(t)$ as follows:

$$\dot{p}(t) = -(p(t)/A) \times k_A(t)u - \lambda p(t) + r(t) \quad (1)$$

$$\dot{m}(t) = (p(t)/A) \times k_A(t)u - (m(t)/B) \times k_B(t)v - \lambda m(t) \quad (2)$$

$$\dot{n}(t) = (m(t)/B) \times k_B(t)v - (n(t)/C) \times k_C(t)w - \lambda n(t) \quad (3)$$

Note, $k_A(t)$, $k_B(t)$, and $k_C(t)$ are radioactivity index to account for the differences in the transportation between day and night. Also, λ is an attenuation coefficient that can be derived from the half-life t_h of ^{32}P.

$$\lambda = \ln 2/t_h \quad (4)$$

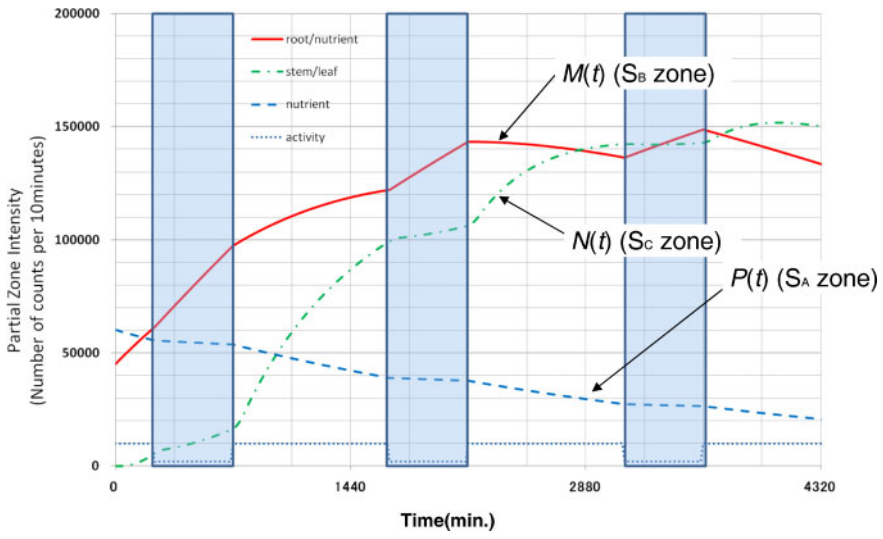

Fig. 5. Absorption simulation using transfer function.

Table 1. Parameters used for ^{32}P uptake simulation.

Items	Numerical values			Remarks
Half life of RI	14.4 days			$\lambda^{-1} = 29\,916$ min
Initial activity of $p(t)$, $m(t)$ & $n(t)$: $p(0)$, $m(0)$, $n(0)$	(Solution) 200 000	(Root) 0	(above) 0	$p(t)$: solution $m(t)$: root $n(t)$: aboveground
Capacity of S_A, S_B & S_C zones: A, B, C	(S_A) 7.0	(S_B) 12.0	(S_C) 2.0	Unit: cm^3
Flow rate between zones: u, v, w	(S_A-S_B) 0.003	(S_B-S_C) 0.003	(S_C-other) 0.003	Unit: cm^3/s
Scale parameter (Scaling): f_A, f_B, f_C	(S_A) 0.25	(S_B) 1.5	(S_C) 10.0	
Mixing ratio: f_p, f_m	0.1	0.9		
Activity index: $k_A(t)$, $k_B(t)$, $k_C(t)$	= 1.0 (on) = 1.0 (off)	= 1.0 (on) = 0.2 (off)	= 1.0 (on) = 0.2 (off)	LED on: 0–4, 12–24 h LED off: 4–12 h

We define $r(t)$ as the back-flow from S_B to S_A *via* activity index $k_B(t)$.

$$r(t) = (m(t)/B) \times (1 - k_B(t))v \tag{5}$$

Through imposing conditions shown in Table 1 to the distribution models, the radioactivity in each part was simulated. Fig. 5 showed the simulated absorption curves in roots and aboveground parts. However, the radioactivity of RI was dependant on the size of the observation area and in the case of the roots, the radioactivity from the culture solution near the roots are counted simultaneously. The numbers $P(t)$, $M(t)$, and $N(t)$ shown in Fig. 5 were scaled against the calculated values $p(t)$, $m(t)$, and $n(t)$ using the following equations:

$$P(t) = f_A \left\{ (f_p p(t) + f_m m(t) \right\} \tag{6}$$

$$M(t) = f_B m(t) \tag{7}$$

$$N(t) = f_C n(t) \tag{8}$$

Note, f_p and f_m are mixing ratios of $p(t)$ and $m(t)$, and f_A, f_B, and f_C are scaling factors used to accommodate three curves derived from Eq. (1) to (3) into a single figure.

The simulation results in Fig. 5 were generally showing the similar tendency to that by experimental data (Fig. 4). The simulation was able to produce the accumulation manner of the phosphoric acid in the roots and the aboveground parts of the plant. However, these mathematical models do not accurately recreate the rapid start of the accumulation seen in the aboveground parts, namely in the stems and the leaves. This suggests that there are more complicated nutrient accumulation mechanism that cannot be expressed with the above mentioned activity index alone. One thing, however, is obvious that during day light the rate of photosynthesis is at its maximum. The aboveground cells would require more phosphorus to produce ATP. This will make the rate of trasnport from roots to stem to increase when the photosynthesis rate is high. If P absorption rate in the roots remains constant at all times it would seem that during the day this rate is lower than at night since P is not staying in the roots but being translocated at a higher rate to the stems.Anyway, further improvements to the mathematical models by considering the nutrients acquisition and accumulation processes would be necessary. Through the experimental results as well as simulation, it is now clear that accumulation manner of phosphoric acid in each area are caused by the different absorption and translocation activity of P during day and night.

5. Summary

Through the development of a real-time RI imaging system that can analyze the radioactivity of RI radiation in a plant sample under controlled condition of light, it has now become possible to record the real-time RI distribution image, and numerical treatment of the image based on radioactivity.

Using *Lotus japonicus* as the sample, we measured the radioactivity of RI over 60 h, and found that the accumulation manner was different between day and night and also among the different parts of the plant. Also, we proposed the differential equations to simulate the accumulation process, and produced radioactivity of each part of the plant. Through this calculation it was suggested that there was a storage capacity of nutrients in roots, as well as aboveground part of the plant, and that the accumulation profiles were controlled by the size of these storage capacities. In future research, we would like to use not only ^{32}P, but also other RI like ^{35}S to analyze transfer mechanisms related to different nutritional elements.

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