Circadian Rhythm in $^{15}$O-Labeled Water Uptake Manner of a Soybean Plant by PETIS (Positron Emitting Tracer Imaging System)

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We present a circadian rhythm of water uptake manner in a soybean plant through realtime imaging of water, labeled with $^{15}$O. Nitrogen gas was irradiated with deuterons accelerated by a cyclotron at Hamamatsu Photonics Co. to produce $^{15}$O-labeled water. Then the $^{15}$O-labeled water was supplied to a soybean plant from the root and the realtime water uptake amount was measured for 20 min by Positron Emitting Tracer Imaging System (PETIS). All the targeting positions for the measurements were stems, two points at an internode between root and the first leaves, between the first leaves and the first trifoliates and between the first trifoliates and the second trifoliates. The water uptake amount was gradually increased and showed its maximum at around 13:00, especially at the basal part of the stem. Then the water uptake activity was gradually decreased until 17:00. The water amount taken up by a plant at 13:00 was about 40% higher than that at 17:00.

Key Words: soybean, oxygen-15-labeled water, water movement, realtime imaging, circadian rhythm

1. Introduction

Circadian rhythm has been known in a plant activity. For example, in leaves of many plants, particularly in the *Leguminosae*, leaves re-orient their laminae photonastically in response to light signals, either to non-directional or directional light signals. These photonastic responses either increase light interception or allow avoidance of damage by excess light$^1)$. During the flower development, circadian rhythm and the role of sugars for photo-induction genes have been reported$^2)$. Since water supports these chemical reactions as a medium, we tried to know whether there was any circadian rhythm in water uptake manner. Though water plays an important role both in chemical processes as well as physical structure, water movement has not been studied in detail, mainly because of the tools for the research are lacking. To measure the realtime measurement of water, we have been trying positron emitting tracer imaging system (PETIS), using $^{18}$F-labeled water$^4), 5)$. With the combination of this method and neutron beam imaging, we could identify water storage tissue in a cowpea plant$^7)$. A cowpea plant was shown to maintain high water uptake activity after drying treatment compared to the other plants, a soybean or a common bean.
However, recently, we found that there was a difference in water uptake activity between $^{18}$F-labeled and $^{15}$O-labeled water. It was our great surprise, for only a trace amount of $^{18}$F was produced from $^{16}$O of water molecule and there was no F$^-$ ion carrier in a target. To scrutinize the PETIS method again, it is important and basic data to know the circadian rhythm of water uptake activity in a plant. Since there has been no report using $^{15}$O-labeled water to measure the change of water uptake manner within a day, through this study, we could choose more accurate timing to measure non-destructive plant activity, especially for water uptake.

2. Materials and Methods

Seeds of a soybean plant (Glycine max c.v. Tsurunoko) were germinated in a wet vermiculite for three days. Then the seedlings were grown in a MGRL water culture solution in a phytotron at 27°C for two weeks under sufficient light with 70% of humidity. The light was on at 7:00 and was off at 19:00. At the measurement, the root of a plant was put into a vinyl bag containing 10 ml of the same water culture solution and was set vertically between the two detectors. The detector was a BGO detector, especially prepared for this experiment. The gamma-rays emitted from $^{15}$O was measured by a pair of BGO (Bi$_4$Ge$_3$O$_{12}$) scintillation detector, whose detection area was 2 mm × 2 mm. Four pairs of the detector were set as shown in Fig. 1. Two of them were set at an internode between the root and the first leaves. The other two were at an internode between the first leaves and the first trifoliates and the one between the first trifoliates and the second trifoliates.

$^{15}$O was produced by the $^{14}$N(d, n)$^{15}$O reaction in a nitrogen gas target containing 0.5% oxygen. The gas was introduced into a target chamber at a rate of 500 ml/min under a pressure of 3 kg/cm$^2$, irradiated with deuteron beams (10 MeV, 15 μA) by a cyclotron (SUMITOMO CYPRIS-HM), and then transferred into an automated radio-synthesizing system supplied by Sumitomo heavy industries Ltd. After the purification of $^{15}$O$_2$ through an ascarite column to remove a $^{15}$O-CO$_2$ component, $^{15}$O$_2$ gas was converted to $^{15}$O-water.
vapor by the platinum-catalyzed reaction of $^{15}$O$_2$ with hydrogen at 150°C. Then, $^{15}$O-water in the solution was prepared by introducing the $^{15}$O-water vapor into a distilled water. After 4 min. irradiation, 1 ml of $^{15}$O-water, about 3 GBq, was supplied to the water culture solution in a vinyl bag with gentle aeration.

3. Results and Discussion

The radioactivity measured by each counter was accumulated every 15 s. Figures from 2 to 5 shows how much water was supplied to each part of the stem, at 12:00, 13:00, 15:00 and 17:00. At each measurement point, reduced radioactivity due to the short half-life of $^{15}$O was calibrated.

![Graph](image1)

**Fig. 2** Water uptake measurement at 12:00. Radioactivity at the position of the stem indicated in Fig. 1 was plotted. The radioactivity was accumulated every 15 s. The position of the measurement was higher in the order of purple, red, yellow and green.

![Graph](image2)

**Fig. 3** Water uptake measurement at 13:00. The measurement was 1 h later than that of Fig. 2.
Though the same amount of radioactive water was supplied to the plant, there is a possibility that not all the positrons emitted from the $^{15}$O were converted to gamma-rays in plant tissue, i.e. there are some escaped positrons which are converted to two gamma-rays outside of the plant, therefore not counted by the detector. We did not calibrate the escaped positrons in the measurement but the rate of these escaped positrons was estimated to be less than 5% (data not shown).

As is shown in Figs. 2 to 5, the radioactivity was higher with respect to the height of the position where the detector was set. Because of an extremely short half-life of $^{15}$O, the experiment was able to perform only for 20 min, ten half-life time of $^{15}$O. Until about 15 min, the plotted data showed a smooth line, but after about 15 min, the plot was somewhat scattered, though the data was...
calibrated based on the half-life of $^{15}$O. Therefore, we had to stop the measurement about 20 min. later. Initially, the water was transferred to the stem closer to the root and gradually went up and only small amount of water was shown at the internode between the first trifoliates and the second trifoliates. There seemed to be a barrier for water transport at the node. When water amount in the first internode and that of the second internode was compared, the water amount in the latter was drastically decreased. When the water amount at the lowest position was taken into account, water uptake activity was the highest at 13:00 and lowest at 17:00 (Fig. 6).

Since the difference of water uptake activity within a day was shown, the measurement timing within a day was found to be an important condition to perform not only PETIS but also in the other experiments to measure a living plant activity.

References

要 旨

PETIS (Positron Emitting Tracer Imaging System) 法を用いたダイズにおける^{15}O 標識水吸水動態の日内変化の測定

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^{15}O 標識水を用い，ダイズにおける標識水吸水動態の日内変化を PETIS (Positron Emitting Tracer Imaging System) 法で測定した。^{15}O 標識水は，サイクロトロンで加速した重水素で窒素ガスを照射することにより調製した。ダイズの根から^{15}O 標識水を供給し，^{15}O から放出されるポジトロンが消滅する際のγ線を1対のγ線検出器で測定することにより，吸水された水の動態のリアルタイム計測を行った。測定時点はすべて室面であり，根と初葉間 2か所，初葉と本葉間，本葉と第二本葉間の 4か所であった。ダイズによる水の吸収は午後 1時が最大であり，午後 5時の場合と比較すると約 40% 高かった。