Water Movement Near the Soybean Root
by Neutron Radiography

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Neutron radiography (NR) was applied to investigate the water movement in soil during the growth of the soybean plant, non-destructively. The plant was grown in a thin aluminum container and was set to the cassette where an X-ray film and a gadolinium converter were sealed in vacuum. Periodically, the sample was taken to the nuclear reactor, JRR-3, installed at Japan Atomic Energy Research Institute. Total neutron flux irradiated was $1.9 \times 10^7$ n/cm$^2$. After irradiation the X-ray film was developed and the sample image was scanned to get the water image. The darkness of the image was corresponded well with the water amount and the resolution was found to be about 15 $\mu$m. Scanning of the image along with the horizontal line showed that much amount of water in the soil was decreased at the part adjacent to the root, compared to that of 1 - 2 mm far from the root. It was also shown that there is the unsymmetrical water uptake of the root at the same depth position. To know the water movement, especially around the secondary root, three dimensional water image was depicted. When the secondary root began to develop, the largest water movement around the primary root was observed especially at the opposite side of the secondary root.

**Key Words**: soybean root, water movement, neutron radiography

**Introduction**

Though neutron radiography (NR) has been shown to be a promising method to know the water movement in plant, there is only a few report in this field$^{1,2}$, at least none in Japan. One of the reason is that there has not been suitable neutron source and facilities available for NR, in Japan. Recently, a nuclear reactor of the Japan Atomic Energy Research Institute, JRR-3, was remodeled and the outlet utilized solely for neutron radiography was set up$^3$. Therefore, the authors are trying to find out in which way the newly set up facility could be used$^{1,3}$. As one of the application of the method, the authors prepared the soybean plant sample to get the water image near the root, non-destructively. Since more than 70% of plant tissue is consisted of water, NR image may provide morphological change of soybean root, as well as water movement in soil.

As to water movement in soil near the root, there has been few report presented based on **in situ** experiment, mainly because of the difficulties to perform the experiment to get the real time data. There are no more than several models to explain the water kinetics around the root. However there are many problems to be solved when these models are verified. The biggest problem is that the plant root is heterogeneous. Since there is no data to evaluate the different root behavior, the actual water movement in soil cannot be estimated by the theory. There has been no suitable method, so far, developed to measure the water...
movement in soil. Therefore, the authors tried non-destructive NR to know the actual water movement near the root. By such NR method, especially real time function and morphological change of plant root, which is still not known well, is expected to be analyzed.

Materials and Methods

The seeds of soybean (*Glycine max. Merril*) were washed well with hypochlorite solution and were germinated till the root length grew about 5 cm, in length. Then the germinating seed was transplanted to an aluminum container (7 mm × 20 mm × 3 mm) and was grown in a phytotron at 25°C, with sufficient light of 20,000 lux. Toyoura's test sand, a silt type with pore size from 197 to 203 μm, containing 18% of water was packed into the thin container. The container was sealed tightly with an aluminum tape, to prevent any water loss due to the direct evaporation from the soil, so that only the water movement by the root uptake could appear in the image. The water was not supplied to the container during the experiment. Then, after 5, 9 and 12 days, the sample was taken out from the phytotron and was prepared for the experiment at the nuclear reactor, JRR-3, of Japan Atomic Energy Research Institute. The plant in the container was fixed to an aluminum cassette where an X-ray film (Kodak, SR) and a gadolinium converter, 25 μm, in thickness, were sealed in a vacuum. The cassette was set vertically and thermal neutron was irradiated.

![NR image of soybean after 5(a), 9(b) and 12(c) day of growth in the container. The darkness in the picture corresponds to the degree of water deficiency. Since water content in root is much higher than that of surrounding soil, the morphological root growth was clearly shown in the image.](image-url)
for 19 s, with the total flux of $1.9 \times 10^7$ n/cm². After irradiation, the samples were highly radioactive due to the formation of $^{28}$Al, half life of which was 2.3 min. About 10 min were needed to cool down the sample for successive processing. At developing the film special care had to be taken. The films had to be stood still while both developing and fixing, in each solution, otherwise the noise was appeared in the image. After the development, the film was scanned (Sharp scanner, JX-325M) to get the positive image. The darkness in the image was corresponded well with the water amount. First, by image analysis, the linear dissection in the soil was performed to know the water distribution, especially, near the root. Next, the three dimensional image, especially around the secondary root was obtained. Then, to know the resolution of the image by the system the authors used, the image was enlarged and the size of one pixel of the picture was measured.

**Results and Discussion**

The root and water image in soil, during the growth, were shown in Fig. 1. The darker part in the figure indicates the water deficient part. Since water content in root was much higher than that in surrounding soil, root shape is clearly shown in the figure. When the container was more than 2 cm, in width, with the same amount of water in soil, the secondary roots were hardly recognized, since, at this width, the amount of water in soil had come to be close to that in root (data not shown). As the root elongated, much water in the soil began to be taken by the root, indicated by the darker soil image in Fig. 1 (c) than Fig. 1.

![Fig. 2](image)

The analysis of the soybean after 5 (a) and 12 (c) day of growth in the container. The image was cut at 6 different position, horizontally. Then the darkness of the image along the line was plotted. The high peak (↑) in the image indicates the root, which did not grow straighly downward in the box. The low peak connected to the high peak indicates the part, where much water was taken by the root. The water uptake was not symmetrically performed around the root, at the same depth position.
To know more about the water movement near the root, the image of (a) and (c) in Fig. 1 were cut at 6 depths, from cut (1) to cut (6), and the darkness in the figure was plotted (Fig. 2). In each dissection plot, the high peak (†) corresponds to the root. Since the root was not growing straightly downward, the root peak in the plot did not appear at the same position of the line. As is shown in the graph, the water uptake near the root, that is the lower peak of the graph, was the highest in cut (1) for both cases, (a) and (c). Cuts (2) and (3) of (a) or (c) show that the water uptake by the root in the same position was not symmetrically performed.

To show the water movement of the soil in more detail, three dimensional water movement, especially around the secondary root was shown (Fig. 3). The images of (a) to (c) in Fig. 3 correspond to those of (a) to (c) in Fig. 1, respectively. As is shown in the figure, the water content in the root was much higher than the surrounding soil, the root shape was expressed as a long high peak. It was interesting that the most water deficient part was around the largest primary root, not around the newly developing secondary root. The darkest part in Fig. 3 (c), which indicated the largest water uptake, was shown around the primary root at the opposite side of the root where the secondary root was grown.

When NR image of the metal with two holes, which are 100 μm apart, was taken, the picture of the two holes were clearly shown as different holes (data not shown).
Fig. 4 The part of Fig. 1(c) was enlarged.
The length of the secondary root was about 1 cm. The size of one pixel composing the image was 15 μm, in length.

Since the metal with holes less than 100 μm apart was not available, the authors tried to get the resolution from the root image presented by a computer (Macintosh IIci). The part of Fig. 1(c) was enlarged and the size of one pixel was measured. Since the color tone of each pixel was clearly different, it was able to get the data based on one pixel. Therefore, from the point to which degree the image could be analyzed, the resolution of the NR image was estimated to be 15 μm, when applying the system the authors used.

Another important factor to regulate the resolution was a nature of n/γ converter. In the experiment, we used the gadolinium converter, which was deposited in a vacuum to be 25 μm in depth. For further experiment, the thickness and the kind of the converter should be investigated. When cold neutron source is available in near future at the institute and NR is performed with cold neutron, the resolution is expected to be one order higher.

The results obtained in the present study indicated that, by NR method, the actual water movement of root-soil system was possible to be obtained, non-destructively. In the experiment, the authors have not been able to get quantitative analysis of the water. Since we are now preparing the computer software to analyze the image quantitatively, the absolute water uptake by the plant from the image might be possible in the near future.

References
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要 旨

中性子ラジオグラフィによるダイズ根近傍の水分動態

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中性子ラジオグラフィを用いてダイズ育成土壌中の水分動態を非破壊状態で調べた。植物はアルミニウムの薄箱の中で育成し、試験的に日本原子力研究所研究炉 JRR-3 に持ち込み、中性子照射を行った。X 線フィルムとガドリニウムのコンバータを真空内蔵させたカセットに試料を固定して照射を行い、中性子ラジオグラフィ像を得た。総中性子束は 1.9×10^7 n/cm² であった。照射後のX 線フィルムを現像し、スキャナでイメージを取り込んだ。像の黒化度は水分量に大きく対応し、像の分解能は約 15 μm であった。像の黒化度から、根から 1 - 2 mm 以内の土壌の水分が最も減少していることが示された。さらに、根の各部分における水分の吸収は非対称であった。二次根周辺の土壌水分の動態をより示すため、像の三次元化を行った。その結果、二次根が発達する反対側の主根部分の水分の動きが最も大きく示された。

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