# A New Method to Estimate Distribution of Crop-Root Activity in Field Soils

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## Background of the present study

Crop-root activity and its distribution in field soils are important factors related to absorption of nutrients and water by crops from soils. Making clear these factors is very useful for fertilizer application technology, for example in designing fertilizer placement and method of soil management. To measure the root activity, $\alpha$-naphtylamine oxidation method or TTC method, both based on the oxidizing activity of roots, and to determine the root distribution a root-system survey method have been employed so far. Although these methods are useful, they have some defects. In the former, there is a problem that the enzyme activity expressed by the $\alpha$-naphthylamine method or TTC method is often not directly parallel to the nutrient-absorbing activity, and in the latter the root system survey method requires much labor, but entire roots can not be sampled, and it is difficult to distinguish degrees of root activity. Furthermore, in all these methods, it is unable to make observations of roots *in situ*, because roots would have to be taken out from soils.

On the other hand, as a method to examine roots *in situ* in field soils, particular pigments or Li, Rb, etc., existing in a relatively small amount in nature, are buried at definite portions of soils, to determine root activity distribution by measuring their absorption by plants. However, this method also has problems that these elements can not easily be identified by conventional method of analysis, and they are not the elements required by plants. Hall et al.[1] improved this method by using $^{32}P$. They buried $^{32}P$ at certain portions of soils, and measured $^{32}P$ in leaves and roots with a certain time interval after planting. By this method, they succeeded in illustrating the root activity distribution of cotton and tabacco. This method is excellent in that P, which is required by plants and which can be measured at a high sensitivity, is used. However, being a method which employs burying, this method also has the following disadvantages: When it takes long time for roots to reach the site of burying either in case of crops with slowly elongating roots, or in case when the site of burying is remote from the plants, even though their roots elongate fast, a strong radioactivity has to be employed due to a short half-life of $^{32}P$. In addition, the soil condition becomes to be different from that of the actual fields due to soil disturbance by the burying.

Nishigaki, Shibuya, and Koyama[2] developed a new method to overcome the defects of the burying method. As the phosphorus absorption of soils is extremely high, phosphate solution with $^{32}P$ label, adsorbed by ion-exchange resin, is injected into soils, using the soil injector shown in Fig. 1, with the purpose of avoiding diffusion loss of the phosphate solution by minimizing its contact with soil, and preventing soil disturbance caused by burying procedure. Root activity can be diagnosed by measuring the $^{32}P$ activity at a certain portion of standing crop plants by the use of a portable GM counter. By this method (which is called "the method to diagnose root activity") root activity of more than 10 species of crops was diagnosed, and the results were utilized to improve cultural practices such as fertilizer placement, time of fertilizer application, period of midsummer drainage to evade serious soil reduction in rice cultivation, or method of planting. However, since this method employs a radioactive isotope, it must be practiced under a regulation of law. But even if the amount of $^{32}P$ to be used is less than the limit of the regulation, and necessary formalities are made, the outdoor use of radioactive substance is almost impossible due to present social and psychological circumstances in Japan.

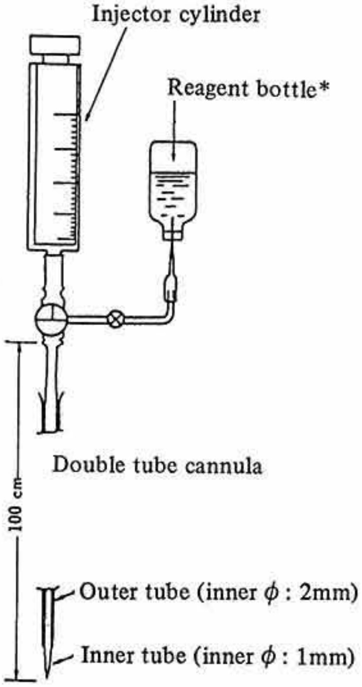


Fig. 1. Apparatus used for injecting a reagent into soils
* Used "penicillin container" is employed as reagent bottle

However, the necessity of diagnosis of root activity distribution is strongly recognized by field research workers. Therefore, the author developed a new method using an activable tracer, that can be employed, completely free from radioactivity, in fields. Outline of the method will be given below.

## Definition of root activity

Root activity mentioned here refers to the function of activity of root itself and density of roots in the soil, and hence defined as root activity per unit soil volume at a given stage of crop growth.

## Principle of diagnosis of root activity

The diagnosing reagent is prepared by using Europium (Eu), a rare earth element rarely existing in nature. The reagent is injected to discretionary solid-geometrical portions of soil with a growing crop. A part (about 50–100 mg) of a definite portion (leaf) of the crop plant which has absorbed Eu through roots is sampled, and Eu in the sample is activated by thermal neutron irradiation. Then, the radioactivity of $^{152}Eu$ is counted by non-destructive method. Thus, from the relative values for absorbed Eu the root-activity distribution can be estimated. This method is an application of so-called activable tracer. The activable tracer method can be carried out completely free from any radioactivity within a tracing experiment system, and hence it is quite useful for outdoor field experiments: Eu used for diagnosing reagent is a non-radioactive element and it is activated only at the time of detection and quantitative analysis of Eu absorbed by plants.

As this method uses a tracer element which is not essential to plant growth, a comparison was made between this method and $^{32}P$ method, and it was confirmed that there is no difference in the pattern of absorption by plants between this method and $^{32}P$ method. Accordingly, it is assumed that root activity shown in absorbing Eu can be applied to the absorption of other nutrients.

## Procedures of the method to diagnose root-activity distribution

*1) Preparation of Eu reagent*

Agar solution of ca. 2%, containing Eu at ca. 4000 ppm is prepared and its pH is adjusted to ca. 6.

*2) Diagnosing method*

By using the injector (Fig. 1), 5 m*l* of the reagent is injected to arbitrary portions of soil, and after 7–14 days, a small amount (ca. 200 mg) of leaf at a definite portion of plant is sampled. The injection is made to 1 portion for 1 plant.

*3) Non-destructive activation analysis*

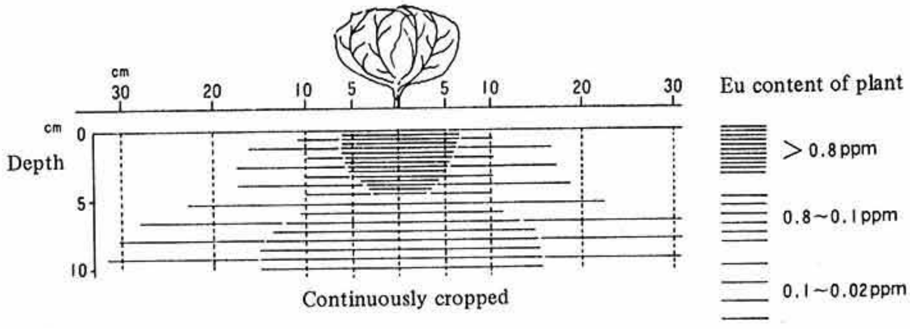
After the leaf sample is air-dried, 50–100 mg of it is taken into a small polyethylene bag, which is then sealed. A group of 20 or 25 bagged samples are placed into an irradiation capsule together with 0.1–1 $\mu$g of standard Eu, and irradiation to thermal neutron (total thermal neutron flux of ca. $5 \times 10^{16}/\mathrm{cm}^2$ is enough). The nuclear reaction occurring by this treatment is $^{151}\mathrm{Eu}\ (\mathrm{n}, \gamma)\ ^{152}\mathrm{Eu}$. The half-life of $^{152}\mathrm{Eu}$ is 13 years, and gamma-ray energy to be used for the measurement is 122 KeV.

The irradiated samples are cooled for a period more than about 3 weeks for the decay of interferring nuclide, and Eu contained in the samples is measured by gamma-ray spectrometry using the apparatus of combined Ge(Li) semiconductor detector and multichannel analyser with the photo-electric peak of 122 KeV of gamma-ray energy as an indicator.

Based on the result thus obtained, root-activity distribution map is produced.

## Root-activity distribution of continuously cropped cabbage

In Inami town, a famous autumn-winter cabbage producing area, of Hyogo Prefecture, cabbage has been grown continuously as a succeeding crop of "early season rice cultivation" since about 1955. As a result of the continuous cropping, it is recognized in recent years that Fusarium yellow disease occurs and yield of cabbage decreases with the repeated continuous croppings unless heavy application of fertilizer is made. To study the reason for decreasing yield, root activity distribution of cabbage was examined in a continuously cropped field and an adjacent non-continuously cropped field, both of them are of the same soil, the same variety and the same method of cultivation (belonging to the same farmer).



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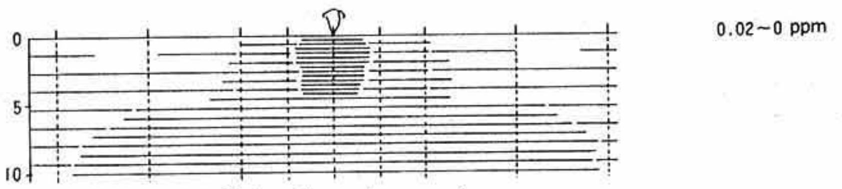

Not continuously cropped

Fig. 2. Root activity distribution map of cabbage

**Table 1.** Concentration of $^{15}N$ and N in cabbage plants to which ammonium sulfate labelled with $^{15}N$ was applied at different placement

| Fertilizer placement | Cropping system | $^{15}N$ atom % excess | N% |
|---|---|---|---|
| Whole inter-row space | Continuously cropped | 0.77181 | 2.97 |
| | Not continuously cropped | 0.92457 | 3.05 |
| Middle part of inter-row space | Continuously cropped | 0.75196 | 2.78 |
| | Not continuously cropped | 0.83513 | 2.99 |

Measurement of $^{15}N$ was made by Dr. Edward Horn, who has been working in cooperation with the present author to develop a method of precise measurement of minute amount of natural $^{15}N$, especially in soils and plants. Apparatus used was MM 602E double collector mass-spectrometer (VG ISOGAS Ltd., England)

At the head expansion stage, the Eu reagent was injected to different soil portions; vertically below the plant and 5, 10, 20 and 30 cm apart from the plant, each at 5 and 10 cm depth from the soil surface. On the 15th day after the injection, a part of the outer leaf was sampled for analysis. The result obtained is given in Fig. 2, which shows an apparent difference in root-activity distribution zone of cabbage between the continuously cropped and not continuously cropped fields. From this result, it is clear that the customary method of fertilizer application, i.e. application to the whole inter-row space, requires heavier application in the continuously cropped field. In other words, the concentrated application to the area within 15 cm from the plants seems to be much more effective in that field, particularly in case of top dressing.

To verify this result, ammonium sulfate labelled with $^{15}N$ was applied to the whole inter-row space or only middle part of the inter-row space, both at the same rate, and $^{15}N$ concentration and nitrogen content were examined with the continuously cropped and the non-continuously cropped cabbages (Table 1). As expected from the above result, the $^{15}N$ concentration in the non-continuously cropped cabbage was consistently higher than that of the continuously cropped cabbage in either method of fertilizer application, and that of the former with the whole inter-row space application was the highest. Namely, the absorption of applied nitrogen was in the order of non-continuous cropping with the whole inter-row space application > non-continuous cropping with the middle part application > continuous cropping with the whole inter-row application > continuous cropping with the middle part application. This order well corresponds to leaf nitrogen contents.

This result offers a clear evidence that effective fertilizer placement is closely related to the root-activity distribution map, and elucidates the reason why the rate of fertilizer application has increased by the continuous cropping of cabbage.

This study on cabbage was conducted as a part of the cooperative research with Mr. Keizo Futami of the Hyogo Prefecture Agricultural Center for Experiment, Extension, and Education.

## References

1) Hall, N. S. et al.: A tracer technique to measure growth and activity of plant root systems. *North Carolina Agr. Exp. Sta. Tech. Bull.*, No. 101 (1953).
2) Nishigaki, S. et al.: Effect of redox-system of soil on activity of rice root. Proceedings of the 2nd Conference on Radioisotopes. 614–615 (1958).

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