We presented alanine, a kind of amino acids, uptake by a rice seedling to study the basic mechanism of the organic fertilizer effectiveness in organic farming. The rice grown in the culture solution containing alanine as a nitrogen source absorbed alanine approximately two times faster than that grown with NH₄⁺ from analysis of ¹⁴C-alanine images by Imaging Plate method. It was suggested that the active transport ability of the rice seedling was induced in roots by existence of alanine in the rhizosphere. The alanine uptake images of the rice roots were acquired every 5 minutes successively by the real-time autoradiography system we developed. The analysis of the successive images showed that alanine uptake was not uniform throughout the root but especially active at the root tip.

Key Words: rice, organic fertilizer, amino acid, ¹⁴C-alanine, real-time autoradiography, direct uptake

1. Introduction

With the increase of an environmental problem such as climate changes, an organic fertilizer derived from recycling activity of the crops and dung in farmland has been gathering the attention. However, the effect of an organic fertilizer differs in kinds of crops, soil condition or even in weather, therefore, it is difficult to evaluate the effectiveness of the organic fertilizer in agriculture. The largest problem in evaluation is that the mechanism or effective chemicals of an organic fertilizer have not been identified.

Nitrogen is one of the most important nutrients for plant growth. In the basic nutritional science, Liebig’s mineral nutrition theory become foundation and reseach of inorganic nitrogen has progressed. That is, plants mainly uptake inorganic nitrogen as nitrate or ammonium ions for the growth. And the available nitrogen supplied from an organic fertilizer is also regarded as inorganic form derived from the decomposition of the organic nitrogen by activities of microorganisms.

On the other hand, in cases of some crops, there is not clear correlation between inorganic nitrogen amount in soil and nitrogen uptake amount in plants¹. Moreover, it was reported that organic form of nitrogen is the main nitrogen source for trees in the forest of north-pole area as well that in the frigid zone like the tundra, where the activities of microorganisms was regulated at low temperature²,³. ¹⁵N, ¹³C-labeled amino acids were used to analyze the direct uptake of organic nitrogen by trees⁴,⁵. However, the study of organic form of nitrogen uptake is very limited compared with that of
inorganic nitrogen. Especially, the study of organic nitrogen uptake from the point of plant nutrient is hardly known, such as uptake manner from soil, metabolic mechanism of absorbed amino acids, environmental condition such as temperature, light and etc. Amino acids are minimum and basic units of proteins or peptides, which are main components of organic nitrogen supplied from an organic fertilizer. Organic nitrogen are decomposed to nitrate and ammonium ions through amino acids by activities of microorganisms. Therefore, studying about the mechanism of amino acids uptake is very important for two reasons. One is to supply a new approach to the plant nutrition research of nitrogen uptake, in addition to inorganic one. The other is for better understanding of organic nutrient supply of organic farming.

In the examination about inorganic nitrogen supplied from the roots, it takes only a few minutes for the nitrate to be transferred from under-ground parts of plant to up-ground by positron emitting tracer imaging system (PETIS). Also in a case of amino acids uptake analysis, it was necessary to label amino acids maintaining the same physical properties and trace labeled amino acids in living plants in real-time. For this purpose, it was the best way to supply radioisotope-labeled amino acids. And we used a conventional imaging plate method (IP FUJIFILM Co.) and the real-time autoradiography system developed by Kanno and Rai, and analyzed the amino acids uptake manner in a rice seedlings from successive images.

2. Materials and Methods

2.1 Growth condition of rice seedlings

Two types of nitrogen pre-culture were performed, one was supplying alanine as a nitrogen source and the other was NH₄⁺. In order to prevent decomposition of amino acids by microorganisms, all of the experiment from germination to developmental stages was carried out under sterilized condition. After sterilized with 0.05% of sodium hypochlorite solution, the seeds of rice (Oryza sativa. cv. Nipponbare) were germinated on the agarose medium at 28 ℃ for 48 hours. Then, some of the seedlings were cultivated in modified Kimura’s culture solution containing 1 mM of alanine and the others were 1 mM of NH₄⁺ as a nitrogen source. The plants were grown in a sterilized plastic boxes, at 25 ℃, under 16 hours of light and 8 hours of dark cycles.

2.2 Imaging plate analysis of distribution of absorbed alanine in a rice seedling

After 12 days from germination, each plant was transferred to the corresponding culture solution containing 0.1 mM of alanine or NH₄⁺ and cultured for 2 days. Then the plant was further cultured for 24 hours in the culture solution containing 1 mM or 5 mM alanine traced with ¹³C·alanine (2.5 kBq / mL, ¹³C·alanine (¹⁴CH₂)₂CH(NH)COOH). Then, the plants were exposed to imaging plates (IP, FUJIFILM, Co.) for 3 days. The images in the IP were scanned by a scanner (FLA-5000, FUJIFILM, Co.) where the resolution was setting as 25 μm. This experiment was repeated three times.

2.3 Real-time imaging of alanine in a rice seedling

After 5 days from germination, the rice seedlings were grown for 7 days in the culture solution containing 1 mM of alanine as nitrogen source. The seedlings were transferred to 25 mL of culture solution containing 0.25 mM alanine culture solution traced with ¹³C-alanine.
(31.7 kBq/mL). The movement of $^{14}$C-alanine was measured by the real-time autoradiography system. Images were taken by integration every 5 minutes successively for 100 hours measurement. By using Aquacosmos (Hamamatsu Photonics, Co.) as software for analysis, we measured with the passage of time about amounts of $^{14}$C-alanine especially in two parts, in the middle part of roots and in the root tips.

3. Results and Discussion

3.1 Imaging plate analysis of distribution of absorbed alanine in a rice seedling

The images taken by an IP were shown in Fig. 1. From these images, amount of the uptake $^{14}$C-alanine in each part of the rice was measured. Comparing the images of the rice seedlings grown under different nitrogen source, the plant grown with alanine absorbed higher amount of alanine than that of NH$_4$$. It suggested that when the rice was grown under the environment, where only alanine was available as a nitrogen source, an ability to absorb amino acids could be expressed. The amount of alanine absorbed by the rice seedlings grown in culture solution containing alanine was approximately 2 times higher than that grown with NH$_4$$. Especially in the shoot, the amount of alanine uptake by the rice seedlings grown with alanine was approximately 3 times higher than that grown with NH$_4$$. These results were similar in both experiments of 1 mM and 5 mM alanine culture solution. The rice seedlings grown with alanine have transferred more alanine from roots to up-ground in higher alanine culture solution (Fig. 2). It was suggested that the active transport ability of rice seedlings was induced in the roots by existence of alanine in the rhizosphere.

![Fig. 1](image1.png)

(A) The rice grown with NH$_4$ as nitrogen source.
(B) The rice grown with alanine as nitrogen source.

Fig. 1 The IP images of alanine distribution in rice seedlings in 5 mM alanine culture solution traced $^{14}$C-alanine.

![Fig. 2](image2.png)

Fig. 2 The influence of different pre-culture on alanine uptake to rice seedlings in 1 mM and 5 mM alanine culture solution traced $^{14}$C-alanine.
3.2 Real-time imaging of alanine in a rice seedling

The successive images of alanine uptake to the roots, acquired by real-time autoradiography system, were shown in Fig. 3. Figure 4 showed that in the root tips, the uptake amount of $^{14}$C-alanine was constantly increased soon after the rice seedling was transferred into the culture solution containing $^{14}$C-alanine. However, in the middle of the root, the amount of $^{14}$C-alanine reached a plateau after 25 hours. It became clear since the rice seedling was under dark condition during the real-time imaging analysis, the transpiration from the leaves was regulated, therefore, the transportation through the vessels was very slow. It was suggested that alanine uptake ability was expressed mainly at the root tip and that middle parts of roots only transported the absorbed alanine.

4. Conclusion

The authors challenged to show that a plant, especially, rice seedlings, absorbs amino acids actively. By taking alanine uptake images by an IP method, we found that the rice seedlings grown in alanine absorbed alanine 2 times faster than the rice seedlings grown in NH$_4$$^+$.

That is to say, the rice seedlings responded to the alanine in the rhizosphere and expressed the new ability of absorption more than usual. Since an IP provided static images of $^{14}$C-alanine distribution in rice seedlings, therefore, we applied a real-time autoradiography system to analyze uptake process of the amino acid. By this system, we got successive images of $^{14}$C-alanine absorbing into the roots. The amount of accumulated $^{14}$C-alanine was higher at the root tips than that in the middle part of the roots with time. It was suggested that alanine uptake ability was expressed mainly at the root
Fig. 4 Changes of $^{14}$C-alanine amounts in different parts of the root in 0.25 mM alanine culture solution traced $^{14}$C-alanine. (A rice seedling grown with alanine as nitrogen source)

tip and that middle parts of roots only transported the absorbed alanine. The living plant’s activity of nutrition uptake, especially in root, has not been known well. Through the real-time autoradiography system we developed, we are going to study the other amino acids movement in living plants more in detail.

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要 旨

イネ幼植物によるアラニンの直接吸収のイメージング解析

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有機農業において有機肥料の肥効がどのように発現しているかを明らかにするため、イネ幼植物によるアミノ酸吸収について解析を行った。13C標識アラニンを吸収させたイネをイメージングプレートで解析した結果、アミノ酸の一つであるアラニンを窒素源として生育させたイネは、アンモニアを窒素源として生育させたイネよりも約2倍早くアラニンを吸収することが明らかとなった。これは根際におけるアラニンの存在にイネが応答し、積極的にこれを取り込むようになることを示している。リアルタイムオートラジオグラフィーシステムを用いて、イネの根がアラニンを取り込む様子を5分間の連続画像として取得した。この連続画像の解析により、イネはアラニンを特に根端において積極的に取り込んでいることが明らかとなった。