

A Study of Glutamine Uptake by Rice Roots

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ABSTRACT

We present uptake and metabolism of glutamine by rice plant roots. Rice plants were cultured in a gel, each containing different kind of 20 amino acids as nitrogen sources under sterile condition. There was a large difference in root development according to the kind of amino acids. Especially in the case of glutamine, the seminal roots and lateral roots were developed well. Therefore, the glutamine absorption manner was analyzed applying ¹⁴C-labeled glutamine, using the real-time imaging system. The uptake amount of glutamine was steadily increased at the root tip. However, in the middle of the root, glutamine uptake curve reached plateau after 10 hours, suggesting that the glutamine accumulation was active at root tip, while the glutamine was only passing through the middle part of the root. To analyze metabolism of glutamine in the plant, doubly labeled amino acids with stable isotopes was applied. In the case of glutamine uptake, the ratio of free amino acids in root was rather constant compared to those when valine were supplied. The mass analysis indicated that absorbed glutamine was metabolized to the other amino acids, suggesting the smooth assimilation of absorbed glutamine.

KEYWORDS: glutamine, rice, root development, uptake, metabolism, real-time imaging system

2. MATERIALS AND METHODS

2.1 Growth of rice plants in different amino acids

A two-day seedling (*Oryza sativa* L.) of rice was cultured with the gel medium containing 5 mM of different kind of 20 amino acids as an only nitrogen source under sterile condition. The plants were grown in a sterilized box for 28 days, at 25°C, under 16 hour light and 8 hour dark condition.

2.2 Real-time movement of glutamine uptake in a rice plant

A two-day seedling of rice was transferred to 20 ml of culture solution containing 0.25 mM ¹⁴C-Gln (18.5 kBq/ml, ¹⁴CO(NH₂)¹⁴CH₂¹⁴CH₂¹⁴CH(NH₂)¹⁴COOH) and the movement of ¹⁴C-Gln was measured by the real-time autoradiography system (Rai et al. 2008). The image was accumulated every 10 minutes and the imaging was performed for 43 hours.

2.3 Amino acid metabolism in a rice plant

Six-day seedlings of rice were transferred to the culture solution containing 1 mM(as nitrogen) Gln labeled with (U)-¹⁵N, ¹³C (¹⁵N, ¹³C 98 atom%, ¹³CO(¹⁵NH₂)¹³CH₂¹³CH₂¹³CH(¹⁵NH₂)¹³COOH) or 1 mM Val labeled with (U)-¹⁵N (¹⁵N, ¹³C 98 atom%, ¹³CH(¹³CH₃)₂¹³CH(¹⁵NH₂)¹³COOH). After 1, 3, 9, 24hours, the plants were harvested and the roots and the up-ground part were separated. Then the amino acids were extracted from the sample with 80% ethanol. The free

amino acids in supernatant were analyzed by liquid chromatograph mass spectrometer (Finigan LTQ, Thermo Fisher Scientific K.K.) .

3. RESULTS AND DISCUSSION

3.1 Growth of rice plants in different amino acids

There was a large difference in root development according to the kind of amino acids supplied (Table.1). Total root length was well when Gln, Asn, Ala, Arg, Asp, Glu was supplied to the root. Especially, in the case of Gln, the seminal roots as well as lateral roots were developed well. On the other hand, inhibition of the root development was shown when Cys, His, Iso, Leu, Lys, Met, Phe, Ser, Thr, Tyr and Val were supplied.

3.2 Real-time movement of glutamine uptake in a rice plant

Successive images by the real-time autoradiography system showed that at the root tip, the uptake amount of ^{14}C -Gln was constantly increased after ^{14}C -Gln was supplied. However, in the middle of the root, ^{14}C -Gln uptake curve reached plateau after about 10 hours, suggesting that glutamine accumulation was active at root tip, while the glutamine was only passing through the middle part of the root.

3.3 Amino acid metabolism in a rice plant

In the case of Gln application, the ratio of free amino acids in root was rather constant compared to those when Val were supplied (Fig.1). Considering mass distribution, absorbed Gln was metabolized to other amino acids, which suggested the smooth assimilation of absorbed Gln. On the one hand, absorbed Val was metabolized to just only Leu. Since the absorbed Gln was estimated to be the starting material for the amino acid synthesis, the utilization of nitrogen from Gln was supposed to induce normal morphological development of roots.

Tab.1 Relative root length of rice seedlings cultured with different amino acids to that without nitrogen application.

Gln	188	Trp	64	Phe	11
Arg	131	Pro	63	Leu	9
Asn	128	NH_4^+	34	Tyr	3
Glu	123	Ile	27	Thr	3
Ala	111	His	14	Ser	3
Asp	86	Cys	12	Lys	3
Gly	77	Val	11	Met	2

100 as cultured by without nitrogen application (Non-N)

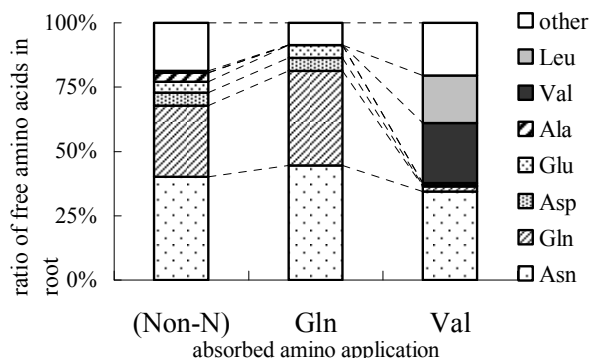


Fig.1 Ratio of free amino acids in root after 24h, when Gln or Val was applied.

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REFERENCES

- Rai H., Kanno S, Hayashi Y, Ohya T., Nihei N., Nakanishi T., 2008 Development of real-time autoradiography system to analyze the movement of the compounds labeled by β -ray emitting nuclide in a living plant. Radioisotope 57(5) 355-360.