3-55 The Production of ^{13}N-labeled Nitrogen Gas for Imaging of Nitrogen Fixation by Soybean Nodule

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The nodule is a symbiotic organ of leguminous plants with rhizobium. Soybean plants utilize nitrogen (N) fixed by nodules from atmospheric N_2. Until now, ^{15}N, a stable isotope, has long been used for kinetics of N_2 fixation and dynamics of transport of fixed N. However, because this method is invasive, it has been difficult to analyze an instant response to environmental (eg. temperature, light conditions) changes with these methods because they are invasive.

We are planning to image and analyze such kinetics and dynamics quantitatively and noninvasively by using nitrogen gas labeled with ^{13}N (half life: 10 min), a positron emitting isotope, and PETIS (positron imaging tracer imaging system). In this paper, we report the synthesis and purification of ^{13}N-labeled nitrogen gas ($^{13}N_2$).

Soybean plants, *Glycine max* [L.] cv. Williams and En1282 (non-nodulated isogenic line) were inoculated with rhizobium (*Bradyrizobium japonicum* strain USDA 110) and were hydroponically cultivated with an N-free medium. The plants were used for the experiment 30 days after sowing.

$^{13}N_2$ was produced with the ^{16}O (p, α) ^{13}N reaction by bombarding CO_2 containing 10% He with proton beam delivered from the TIARA AVF cyclotron. We collected the irradiated gas pressured by Ar gas into a cylinder (Fig. 1, without the tube filled with soda lime). A part of the collected gas was analyzed by Gas Chromatography (G.C.). We found four major peaks in the chromatogram and identified three of them as Ar, $^{13}N_2$, CO_2 by using standard gas analysis (Figs. 2A and 2B), but the fourth peak (indicated as X) has not been identified yet (Fig. 2 B). It is known that more than 10% CO_2 in atmosphere inhibits N_2 fixation [1)], so we tried to remove CO_2 from the irradiated gas. A stainless tube filled with granular soda lime was connected before the collection cylinder. The irradiated gas pressured by Ar gas was collected through this tube into the cylinder (Fig. 1). As a result, the second peak was disappeared. In other words, $^{13}N_2$ without CO_2 was obtained (Fig. 2 C and 2D).

We fed the $^{13}N_2$ to Williams and En1282 and visualized ^{13}N distribution in the roots and nodules. $^{13}N_2$ was mixed with ambient air and fed to the plants for 10 min. After $^{13}N_2$ was washed out with fresh air, the roots and nodules were contacted to imaging plates for the Bio-imaging Analyzer System (FUJIFILM, Tokyo, Japan) for 30 min. We obtained ^{13}N signal in both the nodules and roots near the nodules as shown in Figs. 3A and 3B. However, even in non-nodulated soybean (En1282), approximately 50% signal compared with that of Williams was detected in the roots (Figs. 3C and 3D). We think the signal could be derived from the component X. Therefore, now we are trying to isolate and collect $^{13}N_2$ using G.C. from the irradiated gas.

Reference

1) N. Ohtake et al., TIARA Annual Report 2002 (2003) 114.

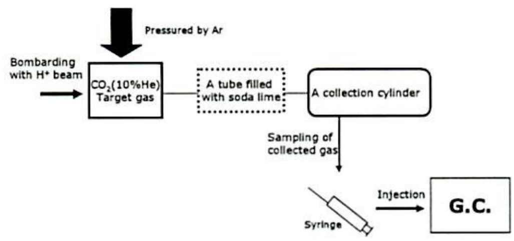

Fig. 1 Scheme of irradiation, collection and analysis of gas.

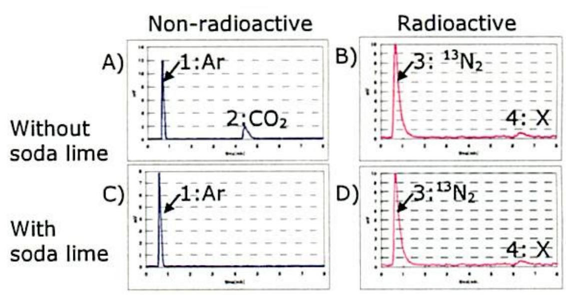

Fig. 2 Gas chromatogram of the collected gas.

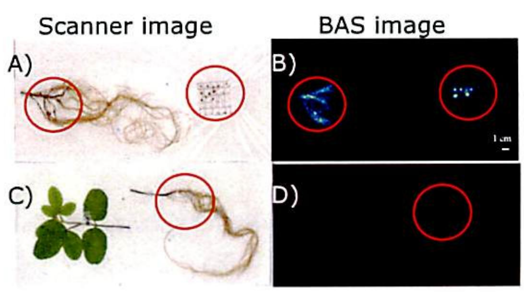

Fig. 3 Scanner and BAS images of Williams (A and B) and En1282 (C and D).