Enhancing nitrogen-fixing capacity of plant-soil system in rice paddy field with low fertility

Laboratory of Crop Science
Department of Plant Production Sciences
Graduate School of Bioagricultural Sciences
Nagoya University
Japan
OKAMOTO, Takanori

Chapter 1: General Introduction

Rice paddies have a high ability to maintain fertility, which is partly due to the ability of bacteria to convert nitrogen (N) molecules in the air into ammonia, a nutrient source for plants, through biological N fixation. Effective utilization of N fixation is expected especially in rice production sites with nutrient-poor soils and inadequate N fertilizer application. In rice cultivation systems in paddy fields, it is reported that N-fixing bacteria living in the rice plant, rhizosphere, and soil contribute to N fixation, and it is considered effective to improve N fixation in a comprehensive manner. N fixation consumes a lot of energy and requires a large amount of carbon source, and it is assumed that the quality and quantity of carbon source in the rice plant and soil affect the N-fixing capacity. It has been reported that the N-fixing capacity of rice is high in the stem and that there is variation in the N-fixing capacity among rice varieties, but the factors and causal genetic factors remain unknown. N fixation in rice stems may increase in response to sugar accumulation, but the main bacterial community of N fixation and the site in the stem remain unknown. It is also known that N fixation requires phosphorus and nitrogenase enzymes contain molybdenum and iron, and that these factors affect N-fixing capacity. In N fixation in paddy soils, it has been reported that fertilization with phosphorus fertilizers, organic matter, and metal-containing materials such as iron can enhance N fixation capacity, but this has not been verified in a wide range of soils, including tropical soils.

Based on these backgrounds, this study focused on N fixation in the stem of rice plants and attempted to elucidate the mechanism of N fixation based on carbon utilization and the composition of the N-fixing bacterial community. I also aimed to clarify the relationship between the N-fixing capacities and the bacterial community. Furthermore, using a set of chromosome segment substitution lines (CSSLs) of *Oryza glaberrima* with high N-fixing capacity, I attempted to identify the chromosomal region responsible for the high N-fixing capacity in the background of *O. sativa*. In addition, I evaluated the effect on N fixation in the soil by applying foundry by-products containing metal elements to paddy field soil. Finally, focusing on Madagascar as a representative example of low-fertility paddy fields, I evaluated the N-fixing capacity of practical Madagascar varieties, and evaluated the effect of the application of locally available compost containing carbon sources and phosphorus on N fixation and analyzed its factors.

Chapter 2: Elucidation of N-fixing bacterial community composition at detailed parts in rice

To clarify the N-fixing sites in the stem, the base of rice stems at the heading stage. The results revealed that the outer part of unelongated stems functioned as higher N-fixing sites. Furthermore, the presence of abundant methane-oxidizing bacteria that utilize methane and sugar-utilizing bacteria such as *Paraburkholderia* sp. in the outer part of unelongated stems suggested that N fixation was performed by these bacteria as they consumed methane and sugar. On the other hand, *Paraburkholderia* sp. and *Sagittula* sp. were the most abundant bacteria in the leaf sheaths, which were the second most active in N-fixing activity, suggesting that they mainly used sugars as substrates. These results indicate that the main bacterial community and their substrates may differ depending on the site.

Chapter 3: Elucidation of rice genotypes with high N fixation capacity

Examination of the N-fixing bacterial community among rice varieties with different stem N-fixing activities and sugar concentrations revealed that the majority of bacterial communities were common among the varieties and were dominated by *Paraburkholderia* sp. and *Bradyrhizobium* sp. On the other hand, *O. glaberrima* variety CG14, which showed high N-fixing activity, tended to have a specifically high abundance of *Kosakonia* sp. To clarify the QTL involved in N-fixing activity, two *O. glaberrima* CSSLs in the genetic background of *O. sativa* were grown and evaluated for N-fixing activity in the stems. The results showed that some lines had higher activity than the *O. sativa* parental cultivar. Chromosome substitution positions of *O. glaberrima*-derived chromosome segments in these lines were located on parts of chromosomes 2, 6, 7, 8, 11, and 12, respectively, indicating the high N-fixing activity of *O. glaberrima* may be controlled by multiple genetic factors.

Chapter 4: Effect of by-products from foundries on N fixation in Japanese paddy soils

The effects of five by-products from the foundry process on N fixation capacity in paddy soils were evaluated by acetylene reduction ability (ARA) and the ^{15}N method in soil culture experiments when applied to paddy soils from three sites in Japan. The results of the evaluation under two conditions, aerobic/light conditions and anaerobic/dark conditions, showed that one material was effective in promoting N fixation capacity by application with cellulose under aerobic/light conditions. The high iron and molybdenum content of this material suggested that these elements may be involved in the promotion of N fixation under aerobic and photoperiodic conditions.

Chapter 5: Evaluation of local rice variety and manure effects on N fixation in rice cultivation system of Madagascar

The rice variety X265, widely used in Madagascar, where nutrient-poor soils predominate, was grown in the field, and its N-fixing activity during the growing period was evaluated using ARA. Total N fixation during the growing period was estimated from the cumulative ARA, and X265 was about four times higher than the Japanese variety Hokuriku 193, indicating that it may be adapted to low-fertility environments and is a promising material for highly active rice varieties. In addition, manure produced in the central highlands of Madagascar from cattle manure, rice straw, local grass, Bozaka (*Aristida* sp.), and soil as primary materials was evaluated for its effectiveness in improving N fixation using paddy field soils from four sites in Madagascar. Soil incubation experiments were conducted under an aerobic/light condition to simulate the topsoil layer and under an anaerobic/dark condition to simulate the subsoil layer. As a result, it was inferred that the carbon source and phosphorus are limiting factors in topsoil, and carbon is major limiting factor in subsoil. The application of manure promoted N fixation to some extent. On the other hand, it was also clear that the manure was not fully decomposed during the period of soil incubation, suggesting the need to establish a method for producing and applying manure that can more effectively improve N fixation, based on the decomposition characteristics of the manure.

Chapter 6: General Discussion

In order to increase N fixation in rice, it is necessary to use varieties with high stem N-fixing ability, such as the Madagascar local variety X265, and to develop rice varieties with QTLs for N fixation derived from *O. glaberrima*. It was also suggested that it is effective to simultaneously enhance root N fixation by methane-oxidizing bacteria base on community analysis. It was suggested that iron-rich by-products of the foundry process may be effective in N fixation, but their use in soils with high iron oxide content, such as those in Madagascar, may cause iron toxicity in rice, so the use of materials that match soil characteristics is required using divers soil types. Agronomic measures to properly supply carbon and phosphorus would be also effective. The development of new varieties with high stem N fixation and manure that can promote N fixation based on the results of this study could expectedly increase N fixation in rice paddies with low fertility, leading to increased rice yield and improved nutrition for farmers.

In conclusion, this study revealed that N fixation in rice stems is higher in the outer part of unelongated stems, suggesting that methane- and sugar-utilizing bacteria fix N there. In addition, bacterial community analysis among rice varieties revealed that while there is a variety-specific community, most bacteria are common among the varieties. Furthermore, I identified chromosomal positions that seem promising for N fixation by *O. glaberrima*. In paddy soils, by-products from Japanese foundries were found to enhance N fixation under aerobic/light conditions. In Madagascar, the local variety X265 was found to have a high N-fixing capacity, and manure application promoted N fixation in local low-nutrient paddy soils. These results suggest that rice and soil N fixation should be evaluated simultaneously in rice paddy fields with low fertility and imply a possibility of further intensifying N fixation capacity.