

## 論文の内容の要旨

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### **Identification of root traits for nitrogen-deficiency tolerance in rice through QTLs analysis**

**(イネの低窒素耐性向上に役立つ根系形質の QTL 解析を用いた同定)**

Rice is one of the most important staple foods not only Asia but also in other regions such as Latin America, because about three billion people, nearly half of the world's population depend on rice for their diet. Nitrogen is an essential major nutrient taken up in large amounts and usually is the most yield-limiting nutrient in rice production around the world. N represents 67% of fertilizer applications to rice on a world basis. However, estimates of the world nitrogen-use efficiency have been calculated to be as low as 33% for cereals. More than half of applied nitrogen fertilizer is lost to the atmosphere or leached into the groundwater and other freshwater bodies causing eutrophication of surface water and contributing to the emission of greenhouse gasses. But farmers applied more nitrogen fertilizers for cultivation to avoid the risk of yield reduction, thus nitrogen fertilizers costs comprise an important fraction of total production costs. Colombia is one of the rice import countries showed higher rice production costs compare to United States and other countries in Latin America since higher cost of nitrogen fertilizer, high amount of fertilizer applications. Thus, improvement of nitrogen-use efficiency should have significant potential both economic and environmental benefits in both low and high input agricultural systems.

Root plays an important role in acquisition of nutrients. But it also responds to different soil environment, may assist plants to scavenge the nutrients in heterogeneous soils. Crop breeders accept that an active root system contributes to enhanced nutrient uptake, resulting in improved grain yield. Thus, improvement of root system architecture is an important breeding target for producing higher yield through improvement of nutrient-acquisition efficiency such as

nitrogen. However, root system architecture showed high degree of plasticity in response to changes different environment in the supply of vital nutrients, and also to environmental cues such as soil moisture, gravity, light and microorganism. Thus, root system plasticity traits are also important breeding strategies to increase water- and nutrient- acquisition efficiency under nutrient-deficiency heterogeneous conditions. However, little is known about interactions between root system architecture trait and agronomic performance under field environments. The objective of this study is to understand the root architectural plasticity changes responded to major nutrient source nitrogen, and root system architectural ideotype in rice to improve agronomic performance under nitrogen-deficient conditions.

To clarify the interactions between root system architectural traits and agronomic performance, we conducted three different experiments at greenhouse and field level from 2012 to 2015, in International Center for Tropical Agriculture (CIAT). For experiments, we have used diverse accessions of both commercial cultivar and wild species of rice. The first experiment is aiming the evaluation of seminal root elongation response to different nitrogen forms ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{NH}_4\text{NO}_3$ ) and concentrations (5, 50 and 500  $\mu\text{M}$ ) by using mesh float method at eight days seedling stage. The result indicated that there is genotypic difference of seminal root elongation response to nitrogen forms and concentrations even at seedling stage. We also found that root elongation in some Latin American commercial varieties such as Curinga was sensitive to nitrogen source, especially  $\text{NH}_4^+$  and concentrations. As increasing of nitrogen concentrations, root elongation of Curinga was inhibited. In contrary to Curinga, root elongation in some wild species such as *O. rufipogon* was insensitive to nitrogen forms and concentrations. Second experiment, we examined the variation in root growth angle and plasticity among rice genotypes grown under hydroponics solution at 40 days old seedling with different  $\text{NH}_4^+$  concentrations using a root basket method. We also observed that there is genotypic variation of rooting pattern response to  $\text{NH}_4^+$ . Especially, rooting pattern as ratio of deeper root in wild species; *O. glaberrima* showed insensitive root growth angle to  $\text{NH}_4^+$  concentrations, in contrary, Curinga also showed sensitive root growth angle.

In third experiment, five contrasting genotypes with distinct rooting patterns (monomorphic shallow, monomorphic deep and dimorphic root system) were selected based on the ratio of deeper root and rooting pattern value from root growth variations at 40 days hydroponic basket method. These distinct genotypes were evaluated to identify the role of root system architecture on plant agronomic performance under paddy field conditions with different nitrogen applications; Colombian farmer's practice (180 kg N / Ha) and native nitrogen conditions (no nitrogen fertilizer application). Nitrogen-deficiency tolerance traits were measured by the ratio of each agronomic performances trait value under native nitrogen to farmer's practice conditions, including grain yield, biomass yield and so on. Dimorphic root system varieties that have both shallow and deep root system showed less yield reduction between native and farmer's practice conditions compare to both monomorphic deep and shallow varieties. We concluded that dimorphic rooting system would be helpful to enhance nitrogen-deficiency tolerance trait of related grain yield under paddy field.

To gain a better understanding about the genetic basis of relationships between root system architecture traits and agronomic performance, we evaluated a set of chromosome substitution segment lines (CSSLs) derived from crosses between two contrast root plasticity genotype; a tropical *japonica* rice cultivar 'Curinga' and a wild species '*Oryza*

*rufipogon*' accession IRGC105491 under three experiments above mentioned with two replications.

Quantitative trait locus (QTL) analysis was conducted with average data of root system architecture traits, agronomic traits and nitrogen-deficiency tolerance traits using CSSL finder v. 0.84 computer program. Following QTLs analysis of each experiment, we identified a total of 18 QTLs; included five QTLs for root system architecture traits on chromosomes 1 and 12, three QTLs for nitrogen-deficiency tolerance on chromosomes 1, 7, 8 and 10 QTLs for agronomical traits on chromosomes 3, 4, 5, 7, 9, 10 and 12 were identified. Even we should be considered undesirable genetic linkage and pleiotropy, the identified QTLs could be used as target region for future breeding because of the possibility of simultaneous improving nitrogen-deficiency tolerance traits.

Interestingly, we found five QTLs on chromosome 1 and a QTL for deeper root number was identified the region of single nucleotide polymorphism marker between id1012330 and id1021697 on chromosome 1 under hydroponic conditions overlapped with a QTL for nitrogen-deficiency tolerance trait of relative grain yield. Although yet we cannot tell that same QTLs may control those two traits, these results suggest that between deeper root trait and grain yield trait there are some relationship and/or recombinant effect. The QTL associated root architecture could potentially be used in future rice-breeding efforts to increase agronomic performance and to maintain grain yield under nitrogen-deficient conditions.

Genetic variations in root system architecture and plasticity of nutrient response may be appropriate targets for marker-assisted selection to improve rice nutrient acquisition efficiency. However, root system architecture is a complex trait that combines root length, root growth angle, root branching number and density of root hair, etc. Recently ideal root system for better agronomical performance under given conditions was proposed. Our challenge is to discover efficient root system architecture that improves nutrient-acquisition efficiency and to identify relevant gene that control ideal root system architecture traits. Future studies in this area are to pyramid useful root system architecture QTLs effectively in single background using advance molecular tools and understanding interaction of genotype x genotype and genotype x environment for the development of rice varieties suitable for nitrogen-deficient conditions in all over the world, especially, Latin America.