論 文 の 内 容 の 要 旨

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論文題目 Functional analysis of rice expressing a Transcription factor, ZmDof1, in response to nitrogen
（トウモロコシ由来の転写調節因子Dof1導入イネの窒素応答に関する機能解析）

Excessive applications of nitrogen fertilizer to maximize crop yields cause negative environmental effects such as pollution and ecological imbalance. To overcome this problem, researchers have attempted to improve the nitrogen assimilation capacity of crops. Maize Dof1 (ZmDof1) is a plant-specific transcription factor shown to promote nitrogen assimilation in *Arabidopsis thaliana* (Arabidopsis) even under nitrogen-deficient conditions. The present thesis shows successful generation of transgenic rice plants expressing ZmDof1 (Dof1 rice), and examines the effects of the introduction of the *ZmDof1* gene on carbon and nitrogen assimilation in rice.

Chapter 1: Generation of transgenic rice plants expressing ZmDof1

Two binary vectors driving ZmDof1 either by cauliflower mosaic virus 35S

promoter or maize ubiquitin promoter (*ZmUbi-1*) were constructed. Transformations into rice calli by *Agrobacterium tumefaciens* were taken place with both constructed binary vectors. However, only transgenic rice plants expressing ZmDof1 driven by *ZmUbi-1* (Dof1 rice) was successfully generated.

Southern hybridization analysis of T_1 generation of Dof1 rice with Dof1-specific probe revealed successful insertions of 1-5 copies of T-DNA. Segregation analysis performed on the T_2 generation showed that single-copy homozygous Dof1 rice could not be obtained. The reason for this outcome is currently unknown, but this result was consistent with the previous study with Arabidopsis (Yanagisawa *et al*., 2004). Thus, it was decided to perform further analyses with T_3 progenies of the Dof1 rice plants harboring *ZmDof1* gene at multiple loci. Three independent lines were selected for further analyses based on seed formation and successful inheritance of *ZmDof1* gene, and these lines were judged to have two copies of *ZmDof1* gene on genome on the basis of the results of Southern hybridization analyses.

Chapter 2: Determination of experimental conditions for analyzing the effects of ZmDof1 expression in rice

There are three general developing phases in rice, vegetative, reproductive, and grain-filling phase. Since it is well known that nitrogen uptake and biomass production during the vegetative phage largely affects yield, it was decided to first evaluate ZmDof1 effects in rice at vegetative stage. The vegetative stage can be further divided into three sub-stages, seedling stage, early and late vegetative stages. In this study, seedling stage, early and late vegetative stages were

considered as 2, 4, and 8 weeks after germination respectively. Effects of ZmDof1 expression were analyzed at each vegetative sub-stage to determine in which growth stage the effects of ZmDof1 in rice would be the most evidently observed. Measurements and comparisons of dry weight as well as nitrogen contents between Dof1 and VC rice plants revealed that the differences between Dof1 rice and VC rice plants became larger as the cultivation length became longer. Based on these results, it was decided to perform more detailed analyses to evaluate ZmDof1 effects in rice at 8 weeks after germination.

Chapter 3: Evaluations of primary characteristics of transgenic rice plants expressing ZmDof1

ZmDof1 induced the expression of phospho*enol*pyruvate carboxylase (PEPC) genes in Dof1 rice plants and transactivated the rice PEPC promoters in protoplast transient assays, showing similar effects in rice as in Arabidopsis. Dof1 rice grown in the presence of 360 μM (sufficient) or 90 μM (deficient) of nitrogen concentrations showed modulation of metabolite contents and gene expressions associated with the anaplerotic pathway for the TCA cycle, suggesting an increased carbon flow toward nitrogen assimilation. One of the most significant alterations observed in Dof1 rice was the increase in asparagine concentrations especially in roots. Furthermore, increases in carbon and nitrogen amounts per seedling were found in Dof1 rice grown under nitrogen-deficient conditions. Nitrogen deficiency also resulted in the predominant distribution of nitrogen to roots, accompanied by significant increases in root biomass and modification of the shoot-to-root ratio. Measurements of the CO_2 gas exchange rates showed a significant increase in the

net photosynthesis rate in Dof1 rice under nitrogen-deficient conditions.

Chapter 4: Effects of ZmDof1 expression in rice onto ammonium assimilation upon recovery from nitrogen starvation

Experiments in chapter 3 showed that ZmDof1 expression in rice enhanced nitrogen assimilation especially under nitrogen deficient condition. However, transcriptional alterations of major genes involved in nitrogen assimilation could not be observed. Therefore it was hypothesized that ZmDof1 effects might be able to be observed when nitrogen assimilation occurs dynamically. Thus, effects of ZmDof1 expression in rice onto ammonium assimilation was monitored by a tracer experiment using stable isotope, ^{15}N. Nitrogen-deficient, two-week old seedlings fed with 90 μM of ^{15}N-labeled ammonium chloride indicated that the rates of initial ammonium assimilation to glutamine were faster in Dof1 rice. As a result, concentrations of subsequent and derivative amino acids of glutamine such as glutamate, aspartate and asparagine, were also found to be significantly higher until 6 hours after nitrogen supply. Contrary to this, concentrations of most amino acids significantly decreased at 48 hours after nitrogen supply, suggesting enhanced protein biosynthesis. These results implied that nitrogen metabolism was not altered abnormally by ZmDof1 expression in rice, but rather the nitrogen metabolism itself was accelerated by ZmDof1 expression in rice, which might enable Dof1 rice for faster nitrogen uptake and/or assimilation.