Regulation of the Expression of Rubisco Small Subunit Genes by Histone Acetyltransferase OsGCN5 in Response

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to Nitrogen Supply in Rice (*Oryza sativa* L.) Xiru Yuan^{1*M2}★, Shicheng Feng², Hayato Ide^{1M2}, Fumiya Miyamoto^{2B4}, Sakae Agarie³, Kazuyuki Saitou³ (¹Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, ²Faculty of Agriculture, Kyushu University, ³Faculty of Agriculture (Graduate school), Kyushu University) イネにおける窒素供給に応答したヒストンアセチル化酵素 GCN5 による Rubi sco 小サブユニット遺伝子の発 現制御 袁夕茹^{1MD★}・馮世程²・井手駿人^{IME}・宮本史也²³⁴・東江栄³・齋藤和幸³ ¹九州大学農学部大学院生物資源環境科学府・²九州大学農学部・³九州大学大学院農学研究院

Introduction: Nitrogen is a primary component of plant substances. Nitrogen deficiency can lead to stunted growth, slow growth, and chlorosis. Five Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) small subunit genes identified in the rice genome are designated as *OsRBCS1,2,3,4* and *5*. Among them, the expression of *OsRBCS3* significantly increases in response to nitrogen. As nitrogen concentration increases, the H3K9/14ac levels of *OsRBCS3* increase, and its transcriptional activity and expression level also greatly increase. It is a histone acetyltransferase that catalyzes this acetylation reaction. However, histone modification events during nitrogen deficiency are not well understood. In this study, we created and selected homozygous transformant rice of *OsGCN5*, GNAT-type histone acetyltransferase, with stable expression levels and investigated the relationship between the expression level of *OsGCN5* and Rubisco small subunit genes.

Materials and Methods: Plants were grown in a plant growth chamber with relative humidity of 70% at 25 °C. During the 6-leaf stage, uppermost fully expanded leaf blades were collected and used for RNA extraction. Quantitative real-time PCR was performed using SYBR[®] Premix DimerEraserTM (Perfect Real Time) (TaKaRa).

Results and Discussion: Transgenic rice plants were generated by using the *Agrobacterium*-mediated transformation method. We created 3 types of transformants which were overexpression transformant of *OsGCN5* under the control of the maize ubiquitin promoter and CaMV 35S promoter, and knockdown transformant of *OsGCN5* by RNAi (*RNAi-GCN5*). We selected 6 individuals of T₀ with low copy number transgene. Accordingly, 5 independent T₁ homozygous lines were obtained. The expression of *OsGCN5* increased in response to nitrogen supply in the wild type. *RNAi-GCN5* caused the decrease of the expression level of *OsGCN5*, and *RNAi-GCN5* transformants grew worse than the wild type. We also found that *OsGCN5* might not regulate the expression of *OsRBCS2* and *OsRBCS4* in response to nitrogen supply in rice. Furthermore, *OsGCN5* positively regulated the expression of *OsRBCS3* was about 10 times higher than that of *OsRBCS5*. In conclusion, *OsGCN5* regulates the expression level of *OsRBCS3* and *OsRBCS5* in response to nitrogen supply in rice.