

Comprehensive studies on functional expression of
OsGAD4 gene in rice plants and physiological analysis
of its genome-edited mutants under abiotic stress
conditions

(要約)

イネにおける *OsGAD4* 遺伝子の機能発現と非生物的不
ストレス下でのそのゲノム編集変異体の生理学的解析に
関する総合研究

AKTER NADIA

Rice (*Oryza sativa* L.) is a crop of utmost importance considering its role as a model plant as well as socio-economic value. For the majority of people on the planet, it is the most common staple food. Though rice is offering energy and basic vital nutrients, its productivity is facing challenges due to abiotic stresses resulting from climate change. GABA (Gamma-aminobutyric acid) is a non-protein amino acid widely known as a major inhibitory neurotransmitter in the central nervous system of mammals. In plants, GABA acts as a signaling molecule and key regulator of growth and development. It is synthesized from glutamate via the enzyme glutamate decarboxylase (GAD). GAD is ubiquitous in all organisms, but only plant GAD has the ability to bind Ca^{2+} /calmodulin (CaM). This kind of binding suppresses the auto-inhibition of Ca^{2+} /calmodulin binding domain (CaMBD) when the active site of GAD is unfolded resulting in stimulated GAD activity. *OsGAD4* is one of the five *GAD* genes in rice genome. It was confirmed that *OsGAD4* can bind to Ca^{2+} /CaM. Moreover, it exhibits the strongest expression against several stress conditions among the five *OsGAD* genes.

In this study, CRISPR/Cas9-mediated genome editing was performed to trim the coding region of CaMBD from the *OsGAD4* gene to remove its autoinhibitory function. DNA sequence analysis of the genome-edited rice plants revealed the truncation of CaMBD (216 bp). The truncated version of the *OsGAD4* mutant showed a significant increment in GAD enzymatic activity in contrast to the wild type. Genome edited line (#14-1) produced 11.26 mg GABA/100g grain, which is almost 9-fold in comparison to the wild type. Short deletion in the coding region for CaMBD yielded in mutant (#14-6) with lower GABA content than wild-type counterpart. Abiotic stresses like salinity, flooding, and drought significantly enhanced GABA accumulation in #14-1 at various time points compared to wild-type and #14-6 under the same stress conditions. Moreover, upregulated mRNA expression in vegetative tissues seems correlated with the stress-responsiveness of *OsGAD4* when exposed to the above-mentioned stresses.

Besides, stress tolerance of *OsGAD4* genome-edited lines was evidenced by the higher survival rate and reduction of biomass loss in terms of fresh weight and dry weight, indicating the gene may induce resilience against abiotic stresses in rice. GABA-enriched mutants were found to have significantly higher antioxidant enzymes such as Catalase (CAT), Peroxidase (POD), and Ascorbate peroxidase (APX) activity, suggesting their ability to detoxify the production of reactive oxygen species (ROS) and thus alleviate the oxidative damage at the cellular level. Moreover, in this study, the severity of 3,3'-diaminobenzidine (DAB) staining or brown spots indicated the degree of H_2O_2 accumulation in the wild-type Ni, #14-1 and #14-6. The intensity of the DAB staining decreased in #14-1 plants under all stress conditions, suggesting the antagonistic mechanism of the line against ROS accumulation.

It can be assumed that GABA has a strongly positive role in regulating the ROS scavenging process

to protect the plant cell from stress. Moreover, RNA-seq analysis reveals a higher number of uniquely expressed genes in the #14-1 line compared to the wild-type which might have a major role in drought stress tolerance. Gene ontology implies cellular components have an influential role in the gene expression process thus speculating the fact that they were majorly associated in stress response pathways.

Additionally, several key genes and gene families associated with drought stress or stress-related conditions were found differentially expressed, including drought-tolerant genes, transcription factors, and signaling protein kinases. Such findings indicate that a high concentration of GABA possibly boosted abiotic stress resilience via modulating gene expression and interconnecting stress signaling and defense systems. It is well known that abiotic stress response in plants primarily involves the regulation of metabolites, reactive oxygen species (ROS) accumulation, and subsequent cell wall damage. GABA actively promotes ROS detoxification in plants, which is accomplished by triggering antioxidative defense mechanisms, though some factors are yet to be explored in plant's stress resistance process. To our best knowledge, this is the first report on abiotic stress tolerance in rice plants modulated by endogenous GABA, opening up new avenues for future research in this field. This research contributes to the field of sustainable agriculture by CRISPR-based approaches that hold significant potential for improving food security and adapting agriculture to a changing climate.