論文の内容の要旨

応用生命化学　専攻
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論文題目

Functional analysis of a drought-responsive gene, *OsbHLHa*, encoding a bHLH transcription factor in *Oryza sativa.*

（イネの bHLH 型転写因子をコードする乾燥ストレス応答性遺伝子 *OsbHLHa* の機能解析）

Chapter 1. Introduction.

Drought is one of the most severe agricultural limiting factors for raising yields. It has been estimated that an additional 100 million tons of crops needs to produce every year, for every one billion people added to the global population. However, water for agriculture worldwide is becoming increasingly scarce. Therefore, it would be very important to develop drought tolerant crops that show increased water use efficiency.

Numerous studies have shown the processes underlying responses to drought stress, especially to severe drought stress, at the cell and molecular levels. In the past twenty years, many genes have been reported to respond to severe drought stress, including some important transcriptional factors like DREBs. Different from the severe drought stress, the mechanism responding to mild drought stress remains mostly unknown. Several reports have shown that the growth of Arabidopsis and rice plants decreased under moderate drought condition, but many of the responding genes were different from those under severe drought stress. Furthermore, the genes in the biosynthesis pathways of phytohormones showed different expression profiles between mild and severe stress conditions. These findings suggest that stress-responsive processes under mild and severe stress conditions are regulated by the different mechanisms.

To better understand the regulation mechanisms that respond to mild drought stress in rice, a soil matric potential (SMP)-based irrigation system, which automatically controls the soil moisture was developed in our laboratory (Todaka et al., 2017). The rice seedlings were planted under different drought levels using this system, and then the seedlings were harvested for the microarray analysis. In the present study, a gene encoding a bHLH type transcription factor, *LOC_Os01g01840* (named *OsbHLHa*), was selected from the differentially expressed genes (DEGs), which exhibited different expression profiles under different drought levels. Molecular characteristics of the gene were investigated using transgenic rice plants overexpressing the *OsbHLHa* and loss-of-function mutants. The biological function of the *OsbHLHa* was examined under different levels of drought stress.

Chapter 2. Identification and characterization of *OsbHLHa*

To screen the DEGs under different levels of drought stress, the transcriptome analysis was performed. Among the DEGs (Fold change ≥3.0), a transcription factor gene, *LOC_Os01g01840*, was selected. Expression of this gene was up-regulated 2.3-fold under mild drought condition denoted Md2 with the SMP value set to -31.0 kPa but down-regulated under severe drought condition, Sds under which irrigation was completely stopped and the SMP was less than -1000 kPa. This gene was named as *OsbHLHa.* Its open reading frame (ORF) was consisted of 690 nucleotides, encoding a peptide with 230 amino acids. Neighbor-joining (NJ) phylogenetic analysis showed that the molecular functions of the nearest homolog genes have not been reported. Quantitative RT-PCR (qRT-PCR) showed the increased expression of the *OsbHLHa* gene under the Md2 and Md3 (with the soil matric potential set to -309.9 kPa) conditions and the decreased expression under the Sds condition. The transient expression assay using rice protoplasts showed that OsbHLHa proteins were localized in the nucleus, indicating that it could play a role as a transcription factor. Tissue specific-expression analysis using transgenic rice plants expressing the *GUS* reporter gene driven by the *OsbHLHa* promoter displayed that this gene was expressed mainly in the young leaves of rice seedlings, in the node and in the

leaf-sheath pulvinus.

Chapter 3. *OsbHLHa* regulates shoot growth

The transgenic rice plants overexpressing the *OsbHLHa* driven by the ubiquitin promoter (OX plants) and the transgenic rice plants expressing the *OsbHLHa* fused to the repression domain driven by the ubiquitin promoter (RD plants) were developed. The OX rice plants represented a semi-dwarf phenotype under well-watered condition compared to the vector control rice plants. Furthermore, the leaf width, seed size, seed weight and seed numbers of the OX plants were decreased compared to those of the control plants. On the contrary, the leaves of RD plants displayed wider compared to those of the vector control plants under well-watered conditions. The numbers of effective tillers, seed numbers, total yields and biomass of RD rice plants were increased compared to those of the vector control ones. These results suggest that the *OsbHLHa* is involved in the regulation of leaf growth and effective tiller numbers.

Chapter 4. *OsLOX11* was the downstream gene of *OsbHLHa*

Microarray analysis was performed using the OX and vector control rice seedlings. The transcriptome analysis identified 175 differentially expressed genes (DEGs) in the OX plants, including 150 up-regulated genes (FC≥2.0, FDR<0.05) and 25 down-regulated genes (FC≤0.5, FDR<0.05). Among the up-regulated DEGs (FC≥2.0, FDR<0.05), two lipoxygenase (LOX) genes *OsLOX11 and OsLOX12* were selected for further analysis as the enzyme has been widely recognized as a critical regulator of the biosynthesis of jasmonic acid (JA). The results of qRT-PCR showed that *OsLOX11* was up-regulated under Md2 and Md3, whereas the gene was down-regulated under Sds, which was consistent with those of the *OsbHLHa*. Transactivation assays were performed using rice protoplasts. The *OsbHLHa* gene driven by the ubiquitin promoter was used as an effector. The activities of the *GUS* reporter genes driven by the -400 bp, -2000 bp and -3000 bp promoters of *OsLOX11* with the effector were significantly increased 2.3-folds, 2.5-folds and 4.7-folds higher than the activity with the empty vector, respectively.

These results suggest that the OsbHLHa functions as a transcriptional activator and increases the expression of *OsLOX11*.

Since it was predicted that the OsLOX11 functions in the JA biosynthesis pathway, the contents of phytohormones including JA-related molecules were analyzed. The contents of JA-isoleucine in the OX plants were significantly higher than the contents in the vector control plants under the Sds condition, although the contents were not different under well-watered condition between the OX and vector control plants. These results imply that the precursors of JA might be involved in the shoot growth retardation of the OX plants.

Chapter 5. Conclusion.

In the present study, the gene *OsbHLHa* was isolated and characterized. *OsbHLHa* showed different expression patterns under different levels of drought conditions. The morphological appearance of transgenic rice plants overexpressing *OsbHLHa* and the loss-of-function mutants indicated that *OsbHLHa* is involved in the regulation of rice growth. Transcriptome and qRT-PCR analyses identified the *OsLOX11* as one of down-stream genes of the OsbHLHa. By the transient transactivation experiment, it was indicated that OsbHLHa functions as a transcriptional activator and increases the expression of *OsLOX11*. The measurements of phytohormone contents suggested that precursors of JA might be involved in the shoot growth retardation of the OX plants. Collectively, it is expected that the biological function of *OsbHLHa* is involved in the shoot growth regulation under mild drought conditions by accumulating the precursors of JA. Biotechnological improvement by utilizing the *OsbHLHa* might overcome the shoot growth retardation under mild drought conditions.

Publications:

Todaka D, Zhao Y, Yoshida T, Kudo M, Kidokoro S, Mizoi J, Kodaira KS, Takebayashi Y, Kojima M, Sakakibara H, Toyooka K, Sato M, Fernie AR, Shinozaki K, Yamaguchi-Shinozaki K (2017) Temporal and spatial changes in gene expression, metabolite accumulation and phytohormone content in rice seedlings grown under drought stress conditions. *Plant J* **90:** 61-78