論文の内容の要旨

応用生命化学　専攻
平成23年度博士課程　入学
氏名　　文　辰錫
指導教員名　篠崎　和子

論文題目

Analysis of the transcriptional regulation of Arabidopsis *PIF* family genes in response to abiotic stresses

(環境ストレス応答におけるシロイヌナズナ*PIF*遺伝子の転写制御機構の解析)

Chapter 1. Introduction

Drought is known as one of the most severe stress conditions that strongly affect the plant growth and productivity. Recent studies indicated that the reduction of plant growth under drought condition is result of adaptation to the stress. Expression of many genes is up-regulated or down-regulated during drought stress condition, and some of these genes were reported to be involved in regulation of plant growth.

As light is an essential environmental factor for growth and development, plants have various kinds of photoreceptors. Phytochromes are representative photoreceptors that are sensitive to red and far-red light. In Arabidopsis, a red light receptor phytochrome B (phyB) can be raised as a well-characterized member of phytochromes known to control photomorphogenesis such as inhibition of hypocotyl elongation and expansion of cotyledon under red light condition by managing the light-signaling network. Members of the PIF (Phytochrome Interacting Factor) family of bHLH transcription factors function in the light-responsive development of chlorophyll, shade avoidance and phytomorphogenesis.

A rice PIF-like gene, *OsPIL1* was identified by microarray analyses as one of the stress-responsive transcription factor genes that were down-regulated by drought stress. The level of the *OsPIL1* mRNA in rice seedlings grown under non-stressed condition with light/dark cycles oscillates in a circadian manner with peaks in the middle of the light period. The expression of *OsPIL1* during the light period is repressed under

drought and low-temperature conditions. OsPIL1 regulates expression of downstream cell wall-related genes and is involved in height of rice plants by changing the internode cell size. However, a mechanism of down-regulation of *OsPIL1* expression under stress condition is not clearly identified yet.

This study aimed to elucidate the mechanism of down-regulation of PIF/PIL family genes under stress conditions in plants by using the most homologous *PIF*/*PIL* genes of *OsPIL1* in Arabidopsis. Down-regulation of the Arabidopsis *PIF* genes was confirmed and their promoter regions were analyzed using transgenic Arabidopsis plants to identify *cis*-acting elements that regulate the expression of the genes under both circadian and drought conditions. The DNA-binding proteins that interact with the identified *cis*-acting elements were tried to isolate using a yeast one-hybrid screening system.

Chapter 2. Identification and characterization of *PIF4* and *PIF5* as the closest orthologs of *OsPIL1*

To identify the orthologous proteins of OsPIL1 in other plant species, the peptide sequences of the DNA-binding bHLH domain conserved in PIF family proteins was used in the phylogenetic analysis. PIF4 and PIF5 were found to be the most orthologous proteins of OsPIL1 in Arabidopsis.

Next, the expression of *PIF4* and *PIF5* genes was verified under drought and low-temperature stress conditions. When Arabidopsis plants grew under non-stressed condition, *PIF4* and *PIF5* expressed in response to diurnal cycles with a peak in the middle of the light period, and their expression was decreased when drought or cold stress started in the light or dark period. As the down-regulation of *PIF4* and *PIF5* in response to drought stress was occurred even in constant light condition, it raised the possibility that the response to drought stress is circadian-independent. The expression of *PIF4* and *PIF5* showed no significant response to other stresses such as high salinity (NaCl), ABA and mannitol treatments, indicating that the down-regulation of *PIF4* and *PIF5* expression is not affected by ABA and osmotic stress.

In transgenic plants expressing a *GUS* reporter gene fused to promoter fragments of *PIF4* and *PIF5*, the *GUS* expression was suppressed in response to drought, low-temperature and diurnal cycle, suggesting that these responses are controlled in the transcriptional level. In order to identify the regulatory regions of the *PIF4* promoter,

truncation analysis was carried out. The region from -288 to -163 of the *PIF4* promoter was found to be positively involved in its expression in the light period. Also, the region from -163 to -70 was found to be involved in negative regulation of *PIF4* expression in response to drought and dark. Further truncation analysis of the region from -130 to -70 was indicated that the region from -117 to -90 is involved in down-regulation under drought condition, and both the regions from -130 to -117 and from -90 to -70 play a role in down-regulation of *PIF4* expression during the dark period.

To figure out the relation between down-regulation of *PIF4* and *PIF5* expression and component of the circadian clock, the expression of *PIF4* and *PIF5* under drought condition in Arabidopsis mutant plants with disrupted circadian clock was analyzed. The expression of *PIF4* and *PIF5* was still down-regulated in the mutants under drought condition, but the down-regulation was mild compared to that in the wild-type plant. These results indicate the partial involvement of circadian components in the down-regulation of *PIF4* and *PIF5* under drought condition, and the existence of other repression mechanisms related to down-regulation of *PIF4* and *PIF5*.

Chapter 3. Isolation and characterization of the HB5 protein that interacts with the regulatory promoter region of *PIF4*

To find interacting factors of the identified regulatory regions of the *PIF4* promoter, the yeast one-hybrid screening was performed using the -288 to -163 region of the *PIF4* promoter as bait and HB5, a member of the HD-Zip I subfamily, was isolated. By phylogenetic analysis of HB5 orthologs from Arabidopsis, rice, soybean and maize, HB6 and HB16 of Arabidopsis were found to be the closest homologs of HB5, and they also showed interaction with the *PIF4* promoter region from -288 to -163. Additionally, the direct interaction of HB5 with the region of the *PIF4* promoter was confirmed by using gel shift assay.

GUS histochemical analysis was carried out using transgenic plants with a *GUS* reporter gene driven by the promoter of *PIF4*, *PIF5* and three *HB* genes. The *GUS* reporter gene driven by the *HB5* promoter was strongly detected in mature leaves, and rather weakly in newly formed leaves, similar to that driven by the *PIF4* and *PIF5* promoters. The *GUS* gene driven by the *HB6* and *HB16* promoters showed rather weak expression in leaves compared to *HB5*, but significantly strong expression in roots. The response of the three *HB* genes to drought stress and diurnal cycle was analyzed by

using quantitative RT-PCR. All three *HB* genes were expressed constitutively in diurnal cycles and the expression of *HB5* and *HB16* were clearly down-regulated in response to drought stress. In Arabidopsis mesophyll protoplasts expressing the *HB* genes fused to s*GFP*, GFP fluorescence of all three constructs was detected specifically in nuclei of the protoplasts.

To verify the transcriptional activities of HB5, HB6 and HB16 on the expression of the *PIF4* promoter, transactivation assay was performed using Arabidopsis protoplasts. HB5 significantly increased the expression level of the reporter gene fused to the *PIF4* promoter and HB6 and HB16 slightly activated its expression, suggesting that these three HB proteins especially HB5 function as positive regulators of the expression of *PIF4*. To further elucidate function of the HB5 protein, transgenic Arabidopsis plants were generated overexpressing *HB5* (HB5-OX plants) or expressing HB5 fused to a repression domain to convert a transcriptional activator into a transcriptional repressor and to overcome potential functional redundancy of the HB6 and HB16 homologs (HB5-SRDX plants). These transgenic plants were analyzed concerning the phenotypes and expression of the *PIF* genes. No significant changes of phenotypes and *PIF4* expression were observed in the HB5-OX plants compared to the vector control plants. In contrast, the HB5-SRDX plants showed dwarf and early-flowering phenotypes and the expression of *PIF4* and *PIF5* was suppressed in the HB5-SRDX plants mainly during the light period.

Chapter 4. Conclusion

In this study, *PIF4* and *PIF5* were found to be the closest orthologs of *OsPIL1* in Arabidopsis, and their expression was also down-regulated in response to drought and low-temperature. Positive and negative regulatory regions of the *PIF4* promoter were identified for its expression under both circadian and drought conditions. The involvements of circadian components and other repression mechanisms were indicated in the down-regulation of *PIF4* and *PIF5* under drought condition. HB5 was isolated as an interacting factor of the regulatory region of the PIF4 promoter and found to be a potential activator of expression of *PIF4*. Further analyses for identification of transcription factors that function in the down-regulation of *PIF4* enable us to figure out the mechanisms of down-regulation of the *PIF* genes under drought stress condition.