論文の内容の要旨

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論文題目

Studies on abiotic stress-responsive genes in pearl millet (*Pennisetum glaucum* (L.) R. Br.)

（トウジンビエの非生物的ストレス応答遺伝子に関する研究）

The plant survival, growth, and productivity were mainly negatively influenced by abiotic stresses, such as drought, high salinity, and high temperature. With the population increases and the environmental changes, the adverse influence of abiotic stresses becomes more serious, which posed serious threats to food security. Improving the abiotic stress tolerance capacity of crops is necessary to increase agricultural productivity to cope with such conditions. Pearl millet (*Pennisetum glaucum* (L.) R. Br., syn. *Cenchrus americanus* (L.) Morrone), the sixth most economically important C4 cereal crop with nutrient-rich seeds, is a good crop to select stress-responsive genes for breeding stress-tolerant crops, as well as to better understand the stress tolerance mechanisms in plants by functional analysis of stress-responsive genes. Therefore, functional analysis of two stress-responsive genes, *PgPM19* and *PgsHsp17.6*, and an important stress-related transcription factor family, *SQUAMOSA* promoter binding protein (SBP)-like transcription factor family was performed in this study.

A pearl millet plasma membrane protein (PgPM19) promotes tolerance to salinity stress in *Arabidopsis thaliana*

One gene, *PgPM19*, which encodes a plasma membrane protein was isolated from pearl millet in this study. The full length of *PgPM19* is 639 bp, which includes 519 bp coding DNA sequence and 120 bp intron region. The encoded protein was predicted as a stable and hydrophilic protein with 4 alpha-helix regions. Consistently, 4 transmembrane domains were also detected, suggesting that PgPM19 might be a membrane-spanning protein. No typical signal peptide was identified and PgPM19 was found to localize in plasma membrane based on the subcellular localization analysis. The expression level of *PgPM19* was highly induced by drought, salinity, heat, and abscisic acid (ABA) in both leaf and root tissues. *PgPM19* overexpressing yeast did not show a significant difference in phenotype with

control under all stress conditions, including mannitol, sorbitol, and NaCl. However, *PgPM19* overexpressing Arabidopsis showed enhanced salinity stress with the highest fresh weight under salinity stress compared with WT and one T-DNA insertion line of Arabidopsis *pm19L1*. *pm19L1* showed decreased salinity stress with lowest fresh weight compared with WT and *PgPM19_OE#1*. Although *PgPM19* was predicted as a target of *PgmiR5082*, the expression analysis between *PgPM19* and *PgmiR5082* showed that the expression of *PgPM19* may not be regulated by *PgmiR5082*. Transcriptome analysis results showed that around 3000 differential expression genes (DEGs) were found in *pm19L1*, WT, and *PgPM19_OE#1* compared with control condition. Among these DEGs, 1013 and 463 unique DEGs in *PgPM19_OE#1* and *pm19L1* respectively, suggesting that both the presence of salinity stress and *PM19* significantly affect the expression levels of related genes. The DEGs in *pm19L1* and *PgPM19_OE#1* are involved in different biological process pathways and only 3 DGEs which showed an opposite expression pattern in *PgPM19_OE#1* and *pm19L1*, raising the possibility that the functions of *PM19* in pearl millet may be different with Arabidopsis PM19L1. Many stress-responsive and ABA-responsive *cis*-elements, such as DRE and ABRE, were found in the upstream 2 kb promoter region of *PgPM19*. Many ABA-responsive genes, including *PYL13*, *PYL6*, *SnRK2.7*, *DREB*s, *ERF*s, *ABI*s, *RAB18*, *RAB28*, *RD29A*, and *RD29B*, were found to be downregulated in *PgPM19_OE#1* compared with WT under salinity stress based on transcriptome data and qRT-PCR validation, indicating that the expression of *PgPM19* is regulated by ABA signaling pathway to confer to salinity stress tolerance.

PgsHSP17.6, a small heat shock protein in pearl millet involved in heat stress responsiveness

Small heat shock protein (sHsp) family genes are well known as molecular chaperones to protect proteins from being denatured in adverse environmental conditions. Based on previous study, 15 members which were highly induced by various abiotic stresses were found in cytosolic class one sHsp gene family. Firstly, the chromosome position, conserved domain, conserved motifs, phylogenetic analysis, and the upstream 2 kb promoter of 13 genes in this family were analyzed in this study. The results found that *PgsHsp16.9B*, *PgsHsp17.1*, and *PgsHsp17.0C* are same gene but not three genes. In this study, one pearl millet gene, *PgsHsp17.6*, who encodes an unstable and hydrophobic protein without a signal peptide. The prediction of secondary structure showed the highest number of extended strands (94) followed by random coils (33) and alpha helixes (29). Then they were grouped into 6 regions presented in the predicted secondary structure and a hollow bar 3D structure. The expression pattern of *PgsHsp17.6* is similar in leaves and roots under many abiotic stresses, including drought, salinity, cold and heat. Heat stress can significantly increase

the expression level of *PgsHSP17.6* in leaf (the fold change up to 189 times) and root (the fold change up to 145 times), while cold stress can significantly decrease the expression level of *PgsHsp17.6* in both tissues. Although the induced expression level of *PgsHsp17.6* is not as high as heat stress, salinity and drought also significantly induced the expression of *PgsHsp17.6* in both tissues. After ABA treatment, *PgsHsp17.6* expression was significantly downregulated in leaves, but upregulated in roots. Taken together, these results indicated that *PgsHsp17.6* not only responses to heat stress but also to other abiotic stresses, including salinity, drought, cold, as well as to an important plant hormone ABA, suggesting its potential roles in regulating plant responses to various abiotic stresses and its potential roles in ABA signaling. *PgmiR171* and *PgmiR164c* were predicted to target *PgsHsp17.6*, which showed opposite expression levels in roots with *PgsHsp17.6* under various stress conditions. Overexpression of *PgsHsp17.6* in yeast cells did not show significant influence on drought and salinity stress tolerance, while showed reduced heat stress tolerance. These results indicated that *PgsHsp17.6* plays negative roles in heat stress tolerance. This study provides important information for better understanding the functions of *PgsHsp17.6* in response to heat stress.

Genome-wide investigation of *SQUAMOSA* promoter binding protein-like transcription factor family in pearl millet (*Pennisetum glaucum* (L) R. Br.)

The *SQUAMOSA* promoter binding protein-like proteins (SBPs) represent a family of plant-specific transcription factors which play essential roles in plant growth, development, and stress responsiveness. In this study, 18 putative *SBP*s (*PgSBP*s) were identified in the genome of pearl millet on the basis of the SBP domain. The lengths of these genomic sequences varied from 2082 bp to 12237 bp and the coding sequences ranged from 501 bp to 2964 bp, with the deduced proteins ranging from 166 to 987 amino acids in length and from 10.67 to 94.01 kDa in protein mass. SBP domains have high similarity with each other and most of the PgSBPs have both Zn-1 and Zn-2 as well as an NLS in their SBP domains. Fourteen *PgSBP*s were distributed on 7 chromosomes unevenly, while the other 4 were located on the scaffolds (i.e., non-chromosomal genomic sequences). Moreover, all the *PgSBP*s were clustered into seven groups (I-VII) and all of these groups contain at least one SBP member from each species based on the phylogenetic analysis. Intron/exon structures of the 18 *PgSBP*s were analyzed by their genomic sequences and protein-coding sequences with the number of exons among *PgSBP*s ranged from 2 to 11. Sixteen motifs were also identified for the 18 PgSBPs and the number of these motifs in each PgSBPs varies from 1 to 10. In general, the intron/exon structures and the motif composition were similar between *PgSBP*s within the same groups. *PgSBP*s in groups V and

VII were predicted as the targets of two microRNAs, *PgmiR156q* and *PgmiR529b*. The expression level of *PgSBP*s and these microRNAs showed an opposite pattern, raised the idea that the expression of *PgSBP*s was regulated by *PgmiR171* or/and *PgmiR164c* indicating that these the expression of *PgSBP*s may regulated through *PgmiR171*- and *PgmiR164c*-PgSBP module. Some *PgSBP*s have abscisic acid (ABA)-responsive elements and stress-responsive elements in their promoters. Expression levels of these *PgSBP*s were upregulated by abiotic stresses and downregulated by ABA. Together, this study shows a comprehensive overview of *PgSBP*s and provides vital information for elucidating the biological functions of *PgSBP*s.