学 位 論 文 内 容 の 要 旨

博士（環境科学）　　氏 名 郭 志慧 (GUO Zhihui)

学 位 論 文 題 名

Characterization of photoperiodic genes *Ghd8* and *Ghd7* on flowering time regulation in a mini-core collection of *Miscanthus sinensis*

（ミニコアコレクションを用いたススキにおける開花期制御に関する日長遺伝子*Ghd8*と*Ghd7*の特徴づけ）

The genus *Miscanthus* is a rhizomatous, self-incompatible, C_4 perennial grass with a wide natural distribution, and is closely related to sugarcane (*Saccharum officinarum*) and sorghum (*Sorghum bicolor*). Owing to its environmental adaptability and high yields with low nutrient requirements, *Miscanthus* is regarded as a potential bioenergy crop. Optimization of flowering time is essential to obtain high biomass yield under different environments, and may also impact biomass quality for *Miscanthus*. Controlling flowering will facilitate the hybridization of genotypes from diverse geographical locations and assist the intergeneric crosses, such as between *Miscanthus* and *Saccharum*. Synchronizing flowering time will also be essential for the development of a seed-propagated crop. Flowering regulation in *Miscanthus sinensis,* one of the important species in *Miscanthus*, was so complicated, operated by thermal time but also a photoperiod sensitivity mechanism. Nowadays, *M. sinensis* is identified as a facultative short-day (SD) plant and days to flower is strongly affected by photoperiod, but the genetic mechanism on controlling flowering in *M. sinensis* is poorly understood. The photoperiod regulation of flowering is well known in rice (*Oryza sativa*), and many significant flowering regulatory genes have been evolutionarily conserved in the Gramineae family. Two essential flowering genes in rice were selected for identification in *M. sinensis*. Therefore, the aim of the present study is 1) to identify allelic and deduced amino acid sequence diversity and geographic distribution of two flowering-related genes in a mini-core collection of *M. sinensis*, representing a wide range of flowering responses to photoperiod, genetic groups (population structure) and latitudes of origin, and 2) to analyze gene expression pattern by quantitative real-time PCR (qRT-PCR) to characterize their response to photoperiod.

GRAIN YIELD, *PLANT HEIGHT AND HEADING DATE 8* (*Ghd8*), a major quantitative trait locus in rice, was isolated in *M. sinensis*. The deduced amino acid sequence of *Ghd8* in *M. sinensis* contained a HEME ACTIVATOR PROTEIN

3/NUCLEAR FACTOR- YB (HAP3/NF-YB) DNA-binding domain, which is critical for the transcription factor function of *Ghd8* gene products. Two homoeologous loci were identified, *MsiGhd8A* located on chromosome 13 and *MsiGhd8B* on chromosome 7, with one on each of this paleo-allotetraploid species' subgenomes of *M. sinensis*. A total of 46 alleles and 28 predicted protein sequence types were detected in 12 wild-collected accessions. Several variants of *MsiGhd8* showed a geographic and latitudinal distribution. Gene expression analysis revealed that *MsiGhd8* expressed under both long-day (LD) and SD conditions, and *MsiGhd8B* showed a significantly higher expression than *MsiGhd8A*.

GRAIN YIELD, *PLANT HEIGHT AND HEADING DATE 7* (*Ghd7*) was generated interest through its genetic interaction with *Ghd8* but also a monocot-specific flowering gene. *Ghd7* is evolutionarily conserved in *M. sinensis,* and the CONSTANS, CONSTANS-like AND TIMING OF CAB1 (CCT)- domain protein was preserved in *MsiGhd7*. One homoeologous locus, *MsiGhd7A* located on chromosome 11 in the A subgenome. While multiple *MsiGhd7B* loci, located at chromosome 12 in the B subgenome, were found a repetitive region in the intron. One putative loss-of-function allele, identified in *MsiGhd8B*, was characterized by an eight-base insertion in the first exon, resulting in a frameshift and eventual premature termination of the protein, and entirely lack CCT domain. Both *MsiGhd7* homoeologous genes expressed higher in LD relative to SD, and the mRNA transcript level of *MsiGhd7* was abundant in the early morning under LD.

The expression pattern of *MsiGhd8* frequently peaked at day time, while *HEADING DATE 1* (*MsiHd1*) peaked at night, indicating that MsiGHD8-HD1 complex might form and accumulate at night, subsequently active the transcription of *MsiGhd7* in the morning under LD condition. And this MsiGHD8-HD1 complex potentially induce expression of *FLOWERING LOCUS T* (*FT*)- *like* genes [*CENTRORADIALIS 8* (*CN8*), *CN12* and *CN15*] under SD condition. *MsiGhd7* functioned as one of the upstream genes of *EARLY HEADING DATE 1* (*Ehd1*), which was suppressed to a greater extent in LD. Moreover, mRNA transcriptional level of *CN8*, *CN12* and *CN15* in *M. sinensis* were greatly promoted under SD condition. Thus, *Ehd1* might be one of the upstream genes of these three florigens. The comparison between days to flower and gene expression for each accession indicated that *CN8*, *CN12* and *CN15* affected flowering time in response to day length in *M. sinensis*. Whereas, for *M. sinensis* from high latitude, the SD might also be a signal to induce a dormancy response, which is epistatic to flowering. Taking together, these gene expression patterns for multiple flowering candidate genes characterize possible pathways that modulates photoperiodic flowering-time in *M. sinensis* under LD and SD conditions.

The present study is the first of this kind of report that screened the diversity and geographic distribution of allele and protein variants, but also investigated the gene expression in response to photoperiod in *M. sinensis*. Identifying these two major genes provides a novel perspective on flowering in *M. sinensis* and will accelerate the process to elucidate the flowering regulatory network of *Miscanthus*. Furthermore, it may provide information for the breeder to improve *Miscanthus* varieties as a bioenergy crop.