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Author(s)	郭, 志慧
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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 *Saccharum* and *Miscanthus*. Synchronizing flowering time will also be essential for the development of a seed-propagated crop. Flowering regulation in *Miscanthus sinensis* 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 strongly affected by photoperiod, but the mechanism controlling flowering in *M. sinensis* is poorly understood. The photoperiod regulation of flowering is fairly well known in *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 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 (QRT) PCR to characterize their response to photoperiod.

GRAIN YIELD, *PLANT HEIGHT AND HEADING DATE 8* (*Ghd8*), a major quantitative trait locus in rice, were isolated in *M. sinensis*. The deduced amino acid sequence of *Ghd8* in *M. sinensis* contained a HAP3/NF-YB DNA-binding domain, which is critical for the transcription factor function of *Ghd8* gene products. Two homoeologous loci, *MsiGhd8A* located on chromosome

13 and *MsiGhd8B* on chromosome 7, with one on each of this paleo-allotetraploid species' subgenomes of *M. sinensis* were identified. A total of 46 alleles and 28 predicted protein sequence types were identified in 12 wild-collected accessions. Several variants of *MsiGhd8* showed a geographic and latitudinal distribution. QRT-PCR 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*), a monocot-specific flowering gene, was also isolated in *M. sinensis* and the CCT domain were conserved 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 of the CCT domain. Both *MsiGhd7* homoeologous genes expressed higher in LD relative to SD, and the mRNA transcript level of *MsiGhd7* increased especially in the early morning under LD.

The expression pattern of *MsiGhd8* frequently peaked at day time, while *HEADING DATE 1* (*MsiHd1*) peaked at night, indicated that MsiGHD8-HD1 complex might form at night, subsequently active the transcription of *MsiGhd7* in the morning. *MsiGhd7* functioned as one of the upstream genes of *EARLY HEADING DATE 1* (*Ehd1*), which was repressed to a greater extent in LD. Moreover, florigens are members of the mammalian phosphatidylethanolamine binding protein (PEBP) family, including *FLOWERING LOCUS T* (*FTs*) that move systemically to promote flowering. Three *FT*-LIKE genes (*CENTRORADIALIS 8* (*CN8*), *CN12*, and *CN15*) in *M. sinensis* were greatly induced under SD condition. Thus, *Ehd1* might be one of the upstream genes of these three florigens. The difference in the expression levels of *CN8*, *CN12*, *CN15* for each individual under LD and SD indicated that these three florigens had strong effects on the flowering induction in *M. sinensis*. While, for *M. sinensis* from high latitude, the SD maybe also a signal to induce a dormancy response, which was epistatic to flowering. Taking together, these gene expression patterns for multiple flowering candidate genes characterize one possible genetic regulatory pathway (*Ghd8*/*Hd1*-*Ghd7*-*Ehd1*-*CN8*/*CN12*/*CN15*) that modulates photoperiodic flowering-time in *M. sinensis*.

The present study is the first of this kind of report which screened the diversity and geographic distribution of allele and protein variants, but also investigated the gene expression in response to photoperiod in *M. sinensis*. The identification of these two 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.