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学位論文題名

Assessment of drought tolerance in *Miscanthus* spp. and *Saccharum* x *Miscanthus*
intergeneric hybrids (miscanes)
(ススキ属およびサトウキビ x ススキ属間雑種 (ミスケーン) の耐乾性評価)

This thesis comprises 111 pages, 10 figures, 6 tables, and four chapters, with two reference literatures.

Global warming is disrupting weather patterns, leading to extreme weather events, unpredictable water availability, exacerbating water scarcity. As a result, water scarcity has become a serious problem for agriculture production with reducing crop yield due to increasing drought in the field. Managing limited water resource becomes a pressing issue to human. On the other hand, reduction of greenhouse gas emission is necessary to stop global warming. Renewable energy to replace fossil fuels is urgent issue. Therefore, developing renewable bioenergy is necessary. To avoid conflict of crops between food and energy, the second-generation bioenergy, producing energy by non-food lignocellulose crops, seems to be a better option for bioenergy resources.

Miscanthus, a C₄ perennial rhizomatous grass, has high biomass productivity in marginal lands and expresses high CO₂ fixation in low-temperature conditions, underscoring its potential as a bioenergy crop in cool temperate environments. A single sterile triploid clone of *Miscanthus* × *giganteus* has been adapted for commercial biomass production in Europe and North America, with average 28.7 Mg biomass production per ha. However, drought may restrain productivity of *Miscanthus*. Although *Miscanthus* exhibited higher light-use efficiency and above-ground biomass relative to maize and switchgrass under drought environment, as a potential energy crop, selection needs to be made of drought-tolerant *Miscanthus* cultivars, which have particularly robust production in marginal agriculture land. Therefore, selecting for accessions and developing drought-tolerant *Miscanthus* cultivars are essential to increase the versatility of *Miscanthus* as a sustainable bioenergy crop.

On the other hand, intergeneric hybrids of *Saccharum* and *Miscanthus*, which often named as miscanes, are also considered as a high potential cellulosic bioenergy crop due to their high biomass potential with thick stems. Based on combining key traits from its parents such as high biomass productivity from sugarcane and chilling and drought tolerance from *Miscanthus*, miscanes are considered as a bioenergy crop especially under warm temperate or subtropical regions. Therefore, miscanes could be expected as a valuable lignocellulosic biomass crop and a source of genes to confer

drought tolerance from *Miscanthus* to sugarcane.

The aim of the present study is 1) to screen *Miscanthus* accessions for drought tolerance with the express purpose of identifying superior genotypes to use as future breeding-stock material, and 2) to evaluate drought tolerance and drought-associated gene expression of miscane genotypes and their sugarcane and *Miscanthus* parents genotypes.

A total of 29 *Miscanthus* accessions of East-Asian origin were screened for drought tolerance with two methods, a dry-down treatment at two locations (Hokkaido University, Sapporo and Brigham Young University, Utah, USA) and a system where soil moisture content (SMC) was maintained at fixed levels using an automatic irrigation system in one location (Sapporo). One genotype, *Miscanthus sinensis* “PMS-285”, showed relatively high drought-tolerance capacity under moderate drought stress. *M. sinensis* “PMS-285”, aligned with the *M. sinensis* ‘Yangtze-Qinling’ genetic cluster, had relatively high principal component analysis ranking values at both two locations. Genotypes derived from the ‘Yangtze-Qinling’ genetic cluster showed relatively greater photosynthetic performance than other genetic clusters, suggesting germplasm from this group could be a potential source of drought-tolerant plant material. Diploid genotypes also showed stronger drought tolerance than tetraploid genotypes, suggesting ploidy could be an influential factor for this trait.

Three miscane genotypes, derived from hybridizations between one sugarcane genotype and one *Miscanthus* genotype were evaluated for their drought tolerance and gene expression of three drought-associated genes, which were *Hydroxyacid oxidase 1 (HAOI)*, one stress-responsive NAC genes of *Miscanthus lutarioriparius (MINAC12)*, one mitogen-activated protein kinase gene of rice (*OsMAPK5*). In greenhouse experiments, drought stress index (DSI) of all 3 genotypes of miscane were higher than the sugarcane parent after 7 or 8 days of drought. One genotype, miscane “JM 14-60”, showed high photosynthesis rate and high DSI under drought environment, which is similar to its *Miscanthus* parent genotype. Drought-associated gene expression experiment revealed that *Miscanthus sacchariflorus* “Miyakonojo” and miscane “JM 14-60” genotype expressed higher level for *MINAC12* and *OsMAPK5* in drought relative to control condition. As a result, miscane “JM 14-60” showed relatively high drought-tolerance capacity and it is considered as a superior genotype for introgression breeding programs.

The present study is the first of its kind report which assessed drought tolerance in *Miscanthus* spp. and *Saccharum x Miscanthus* intergeneric hybrids (miscanes) in term of genotypic variability. The drought tolerant genotypes, *M. sinensis* “PMS-285” and miscane “JM 14-60” could be valuable for elucidation of drought stress mechanism and improving drought tolerance in *Miscanthus* spp. and a source of genes to confer drought tolerance from *Miscanthus* to sugarcane in the further studies. Of the two methods, the dry-down treatment appears more suitable for selecting drought-tolerant genotypes given that it reflects water-stress conditions in the field. However, the fixed-SMC experiment may be good for understanding the physiological responses of plants to relatively constant water-stress levels. As mentioned above, the present study could provide an important insight for researchers interested in studying sustainable biomass production of *Miscanthus* and miscanes as bioenergy crops.

Therefore, we acknowledge that Ms. Weng Tzuya is qualified to be granted the Degree of Doctor of Philosophy in Food Resources from Hokkaido University.