学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Chilling tolerance and biomass production of *Saccharum* × *Miscanthus* intergeneric hybrids (miscanes) in cool climatic conditions of northern Japan
(北日本の冷帯気候条件でのサトウキビ属×ススキ属の属間雑種（ミスケーン）の低温耐性とバイオマス生産)

Biomass is the most common form of renewable resource. In 1992 at the Rio United Nations Conference on environment and development, the renewable intensive global energy scenario (RIGES) suggested that, by 2050, approximately 50% of the world's current primary energy consumption, could be met by biomass and 60% of the world electricity market would be supplied by renewable sources of which biomass is a significant part. Biomass is produced by green plants that convert sunlight into plant material through photosynthesis. 'Dedicated biomass energy crop' refers to nonfood crops that are solely grown for biomass production. Sugarcane (complex hybrids of *Saccharum officinarum* L., *Saccharum robustum* Brandes & Jeswiet ex Grassl, *Saccharum spontaneum* L., *Saccharum barberi* Jeswiet and *Saccharum sinense* Roxb. amend. Jeswiet), which is cultivated on 26.7 mha and yields nearly 1.9 billion metric tons per year with a peak dry matter yield >100 tons (ha yr^{-1}), is among the highest biomass-producing crops in the world. In addition to being the leading sugar-producing crop for human consumption, sugarcane is also used as a lignocellulosic biomass and a feedstock for bioethanol production. However, the lack of environmental adaptation of sugarcane has been a persistent problem, especially in high-latitude and/or high-elevation areas, owing to its susceptibility to cold. Sugarcane is one of the most chilling-sensitive crop species in the world. At temperatures below 20 °C, sugarcane leaf production slows, and below 10 to 15 °C growth ceases completely. Photosynthesis in sugarcane ceases between 8 to 12 °C and severe frost (-5 to -7 °C) can completely kill the aboveground plant. Hybridization programs have been attempted to improve cold tolerance and adaptation of this crop to cooler environments. Crosses between commercial sugarcane (hybrids of mainly *S. officinarum* and *S. spontaneum*) and *S. spontaneum* have been reported to show cold tolerance. However, these crosses are not reliable for cultivation due to their varying degree of chilling tolerance. In addition to *S. spontaneum*, taxa in the *Saccharum* genus can be crossed with related genera belonging to so-called "*Saccharum* complex", which includes *Miscanthus*, *Erianthus*, *Narenga*, *Sclerostachya*, or other genetically similar perennial grasses. Among these related genera to *Saccharum*, *Miscanthus* is receiving a lot of focus in recent years as a potential genetic resource, especially to improve cold-tolerance in sugarcane. *Miscanthus*, a native C_4 grass of East-Asia, unlike sugarcane, shows a high degree of cold tolerance compared to other warm-season, C_4 perennials. *Miscanthus* was reported to produce shoots at a temperature as low as 6 °C and survive after prolonged exposure to temperatures <-6.5 °C. Putative hybrids of *Saccharum* and *Miscanthus* have been studied since the late 1940s for their biomass production and adaptive traits. These hybrids, often termed as miscanes, show potential as lignocellulosic biomass crops with their strong, thick culms, long stems, and high biomass-yield potential. Preliminary studies on miscanes have shown a possible introgression of cold tolerance from *Miscanthus* to sugarcane that could lead to greater biomass production under subtropical and warm-temperate latitudes.

The aim of the present study is 1) to observe genotypic variability in photosynthesis and biomass traits that will ensure important selection criteria to improve sugarcane; 2) to test chilling tolerance in miscane compared to its

parent, sugarcane and *Miscanthus* which will confirm the introgression of chilling tolerance traits into sugarcane from *Miscanthus*; 3) to test the biomass production capacity of miscanes as a potential biomass energy crop in higher latitudes of the world.

Eighteen miscane genotypes, derived from intergeneric hybridizations between two genotypes of sugarcane and two genotypes of *Miscanthus* (one each of *M. sinensis* and *M. sacchariflorus*) were used to screen for chilling tolerance under low temperature. In a greenhouse experiment on long-duration chilling stress (12–13 °C day/7–9 °C night) photosynthetic rates of the *Miscanthus* parents were significantly higher than the sugarcane parents after seven days of chilling and were more than double by 14 days. The *Miscanthus* also retained more of their pre-chilling (22–25 °C day/13–15 °C night) photosynthetic rates (68–72% seven days, 64–66% 14 days) than the sugarcanes (27% seven days, 19-20% 14 days). Seven of 18 miscane genotypes exhibited higher photosynthetic rates than their sugarcane parents after seven days of chilling, whereas after 14 days only four miscane genotypes had significantly higher photosynthetic rates than their sugarcane parents, but notably two of these did not differ from their highly tolerant *Miscanthus* parents. The results indicated variability in chilling tolerance in miscane progenies, thus selection will be a key aspect of improving chilling tolerance in sugarcane.

In three subsequent growth-chamber experiments to evaluate chilling stress and post-chilling recovery, four miscane genotypes representing the range of responses observed in the greenhouse experiment were compared with their parents. After short-term and long-term chilling stresses of different temperatures, the miscanes retained more of their pre-chilling photosynthetic rate compared to their sugarcane parents, with some of the genotypes not significantly different from their *Miscanthus* parent and cold-tolerant *Miscanthus* ×*giganteus*. After one and seven days of post-chilling recovery, the *Miscanthus* genotypes and miscanes fully recovered their pre-chilling photosynthetic rates but the sugarcane parents did not. These experiments confirmed that genes from *Miscanthus* can be used to improve chilling tolerance of sugarcane via introgression.

In addition, field experiment trials evaluated miscane genotypes and their parents for their seasonal variation in photosynthesis and biomass production under field conditions in Hokkaido, Japan to identify promising genotypes and traits, which can be selected to further improve sugarcane. Results showed several of the miscane genotypes have high early- and late-season photosynthesis coupled with high biomass production, likely indicating chilling tolerance. High broad-sense heritabilities for traits, including stem diameter, tiller number, leaf width, leaf and stem dry weight, and high correlations between these traits and dry matter yield indicated that selections can be made efficiently to improve sugarcane. Miscane 'JM 14-09' was identified as a superior genotype for introgression breeding programs and as a potential energycane cultivar for its high biomass-production capacity.

The present study is the first of its kind report which elucidated genotypic variability, chilling tolerance capacity and biomass production of *Saccharum* × *Miscanthus* intergeneric hybrids (miscanes) under warm-temperate climate of northern Japan, and compared them to their respective parental genotypes and taxa belonging to "*Saccharum* complex". Chilling tolerant and high biomass potential under cool temperature were identified in miscanes. Key traits for selection to improve chilling tolerance and biomass were also identified.

The main limitation of the present study was the field observation at the high latitudinal condition of Hokkaido (USDA hardiness zone 7b), hence, the overwintering capacity of miscanes could not be tested. In order to develop actual breeding lines, the future research should test: 1) perennial biomass production capacity of miscanes at lower latitudes compared to Hokkaido where miscanes can successfully overwinter; 2) the feedstock quality aspects such as cellulose, hemicellulose and lignin content for energy value in miscanes. A further study on genetic aspect of chilling-tolerance in miscanes, focused on photosynthetic genes such as Pyruvate phosphate dikinase (PPDK), Ribulose bisphosphate carboxylase/oxygenase (Rubisco) and Phosphoenolpyruvate carboxylase (PEPC), is also needed to get a clearer picture of the molecular mechanism of chilling tolerance observed in miscanes. As mentioned above, the present study could provide an important insight for future researchers interested in studying sustainable biomass production in subtropical and warm-temperate zones.