

学位論文（要約）

論文題目：Study on DNA content variation in Miscanthus species in Japan  
（日本産ススキ属植物の DNA 量変異に関する研究）

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## Summary

*Miscanthus* species are of potential value as bio-resources. Among the available species, Giant Miscanthus (*Miscanthus* × *giganteus*), a highly productive sterile triploid hybrid grass that was discovered in Japan several decades ago, has considerable potential as an alternative source of energy. While, there are many species of wild *Miscanthus* in Japan, there are very few genetic studies. There is high possibility of discovery of new bio-resources in this wild *Miscanthus* species in Japan. Then, I want to know (i) degree of hybrid formation between *M. sinensis* and *M. sacchariflorus* in Japan through analysis of DNA content of mature plants by using flow cytometry because 3x hybrids are potential energy crop, (ii) hybridization rates between 2x *M. sinensis* and 4x *M. sacchariflorus* of seedling from 5 areas of Japan to discover new 3x hybrids as potential energy crop, (iii) variation of DNA content of wild *Miscanthus* species in Japan, such as *M. sinensis*, *M. sacchariflorus*, *M. intermedius*, *M. oligostachyus*, *M. tinctorius*, *M. floridulus* and *M. sinensis* var. *condensatus*.

All sample were assessed DNA content by using flow cytometry (Cell Lab Quanta SC MPL).

1. This study was conducted a comparison of the relative DNA contents estimated with AT-selective DAPI dye and intercalary PI dye in diploid (2x) *M. sinensis* and tetraploid (4x) *M. sacchariflorus* and their triploid (3x) hybrids. A strong linear correlation was observed between both FCM analyses. The slope of the regression line was 1.15. This result shows the high reliability of the findings of previous study on *Miscanthus* accessions.

2. The ploidy level of 2 *Miscanthus* species was determined through using DNA content data of 170 mature plants from 4 sites of Japan and 65 plants from Kushima site. There are no 3x mature plants in Kushima which is the discover site of three triploid seedlings. Totally, no 3x mature plants were found from 235 mature plants in Japan. This result

shows very restricted gene flow between *M. sinensis* and *M. sacchariflorus* in Japan.

3. The ploidy level of 507 seedlings of *M. sacchariflorus* and *M. sinensis* from 5 localities in Japan was determined through using DNA content data. I identified triploid hybrids in seedlings of *M. sacchariflorus* from Gifu, Miyazaki, Kushima and Tsukuba (15, 5, 5 and 4, respectively), but no 3x hybrid of seedlings of *M. sinensis*.

4. The mean DNA contents of *Miscanthus* species were *M. intermedius* (16.53 pg), *M. oligostachyus* (5.80 pg), *M. tinctorius* (5.69 pg), *M. floridulus* (5.19 pg) and *M. sinensis* var. *condensatus* (5.38 pg). It is clear that *M. intermedius* is a 6x species. I also discovered a 4x *M. intermedius* plant. The clade including these polyploidy level taxons were newly recognized by phylogeny study by using DNA sequence data (ITS and cpDNA).

It is expected that these new discovered hybrids would be used as bioenergy crops in the near future.