

Genomic-Enabled Breeding of *Miscanthus*

Lindsay V. Clark¹, Kossonou Anzoua², Larisa Bagmet³, Pavel Chebukin³, Bruce E. Coulman⁴, Elena Dzyubenko³, Nikolay Dzyubenko³, Xiaoli Jin⁵, Douglas A. Johnson⁶, Uffe Jørgensen⁷, Jens Bonderup Kjeldsen⁷, Stephen P. Long¹, Hironori Nagano², Karen Koefoed Petersen⁷, Andrey Sabitov³, Ji Hye Yoo⁸, Chang Yeon Yu⁸, Toshihiko Yamada², and **Erik J. Sacks**^{1*} (esacks@illinois.edu)

¹University of Illinois, Urbana, IL; ²Hokkaido University, Sapporo, Japan; ³Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg, Russia; ⁴University of Saskatchewan, Saskatoon, Canada; ⁵Zhejiang University, Hangzhou, China; ⁶USDA-ARS Forage and Range Research Lab, Logan, UT; ⁷Aarhus University, Tjele, Denmark; and ⁸Kangwon National University, Chuncheon, South Korea

Project Goals: The primary goal of this project is to facilitate the development of *Miscanthus* as a bioenergy crop by obtaining fundamental knowledge about its genetic diversity, population structure, and environmental adaptation. We also seek to identify molecular markers associated with traits of interest to greatly increase the efficiency of breeding improved cultivars. Such knowledge will also facilitate efforts to introgress useful genes for abiotic and biotic stress tolerance from *Miscanthus* into *Saccharum*, thereby bolstering the development of more winter-hardy and disease-free sugarcanes and energycanes than are currently available.

Miscanthus is a genus of C₄ perennial grasses native to East Asia and Oceania, and it is a promising biomass crop for bioenergy and bioproducts. However, for *Miscanthus* biomass production, only one sterile triploid genotype of *M. ×giganteus* has been grown commercially in North America and Europe; this clone was introduced from Japan to Denmark in the 1930s. Thus, there is a pressing need to expand the diversity of *M. ×giganteus* genotypes available to growers. *M. ×giganteus* is derived from *M. sinensis* and *M. sacchariflorus*. Thus, a prerequisite to breeding improved *M. ×giganteus* is to obtain, characterize, and breed diverse germplasm pools of both *M. sinensis* and *M. sacchariflorus*.

Through two parallel DOE Feedstock Genomics projects, we have revealed the population structure of *M. sinensis* and *M. sacchariflorus* throughout most of these species' native ranges in East Asia. From 617 *M. sinensis* genotypes and ~21,000 RAD-seq SNP markers, we identified seven genetic groups (all diploid) via Structure analysis and discriminant analysis of principal components that corresponded to geographically distinct regions. Similarly, from 764 *M. sacchariflorus* genotypes and ~35,000 SNPs, we identified six geographically distinct genetic groups, including two tetraploid groups from Japan, an independently derived tetraploid group from Korea and N China, and three diploid groups. *M. sinensis* shows evidence of a large population bottleneck during the last glacial maximum, but the more cold-adapted *M. sacchariflorus* was less restricted in range and has higher genetic diversity overall.

These population structure studies, combined with data from field trials at multiple locations in Asia and North America, have facilitated the identification of SNPs associated with key traits, such as biomass yield, overwintering ability, and flowering time. The field trials for the *M. sinensis* study have been completed but those for *M. sacchariflorus* are still underway. Notably, dry biomass yield of four *M. sinensis* accessions exceeded 80 Mg ha⁻¹ in Zhuji, China, approaching the highest observed for any land plant. Additionally, six *M. sinensis* in Sapporo, Japan and one in Leamington, Canada also yielded more than the triploid *M. ×giganteus* ‘Illinois’ control, with values exceeding 20 Mg ha⁻¹. For genome-wide association (GWA) analyses, the *M. sinensis* germplasm panel was genotyped with 46,177 SNPs, enabling identification of 27 significant SNPs for yield. Genomic prediction accuracy for biomass yield of *M. sinensis* was 0.47 over five northern sites and 0.65 for our southern location in Zhuji, China. These results have established a baseline of data for initiating genomics-assisted breeding to improve biomass yield of *M. sinensis* and *M. ×giganteus* in a diverse set of relevant geographies. Preliminary results from the *M. sacchariflorus* study indicate that we are making similar gains with that species. Furthermore, the parallel nature of these *M. sinensis* and *M. sacchariflorus* studies, enable genomic comparisons between the two species; to illustrate this point, we present comparative GWA analyses for flowering time, a highly repeatable trait.

Future work, with support from the Center for Advanced Bioenergy and Bioproducts Innovation (a DOE Bioenergy Research Center), will begin to explore gains that can be realized for yield and winter-hardiness by implementing genomic selection within the *M. sinensis* and *M. sacchariflorus* genetic groups that we identified previously. This work will benefit greatly from the *M. sinensis* reference genome released this year by JGI. For example, using the pre-release version of the genome, we have been able to identify hundreds of thousands of SNPs from the *M. sacchariflorus* panel, which is an order of magnitude increase.

Publications

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2. Clark, L.V., E. Dzyubenko, N. Dzyubenko, L. Bagmet, A. Sabitov, P. Chebukin, D.A. Johnson, J.B. Kjeldsen, K.K. Petersen, U. Jørgensen, J.H. Yoo, K. Heo, C.Y. Yu, H. Zhao, X. Jin, J. Peng, T. Yamada, and E.J. Sacks. 2016. Ecological characteristics and *in situ* genetic associations for yield-component traits of wild *Miscanthus* from eastern Russia. *Annals of Botany* doi: 10.1093/aob/mcw137.
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