

Quantifying phenotypic and genetic diversity of *Miscanthus sacchariflorus* to facilitate knowledge-directed improvement of *M. xgiganteus* (*M. sinensis* × *M. sacchariflorus*) and sugarcane

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Project objectives:

- A. Determine population structure for a germplasm collection of ~625 *Miscanthus sacchariflorus* genotypes from throughout the species natural distribution in China, Japan, Korea and Russia to identify distinct and potentially heterotic groups.
- B. Quantify phenotypic variation in *M. sacchariflorus* for key traits (primarily yield, yield-components, flowering time, overwintering ability, chilling tolerance, and low temperature photosynthesis) at field trial sites in the U.S., Canada, Denmark and Asia, and assess the effects of genotype × environment (G×E) interactions, to facilitate the selection of parents for remaking improved versions of *M. xgiganteus* and for introgressing cold-tolerance into sugarcane.
- C. Enable efficient use of *M. sacchariflorus* germplasm by identifying molecular markers associated with key traits, through the testing of candidate genes, and genome-wide association mapping.

Accomplishments, and impacts:**Material exchange & field trial establishment:**

By September 2014, the collaborators on the project signed a single MTA that is consistent with the Convention on Biological Diversity, allowing the participants to share germplasm, and providing the option for further breeding and commercialization via benefit-sharing with providers. In Q1 of 2015, the collaborators in China, Japan, Korea and the U.S. sent dormant rhizomes to each other and to our collaborators in Canada and Denmark.

In total, 848 accessions of *M. sacchariflorus* were collected, representing a broad geography from near 50 N in eastern Russia to southwest China, and from USDA hardiness zone 3 to zone 9 (Table 1, Figs. 1 and 2). Most of the accessions were successfully established and propagated at each of the collaborators' locations in Canada, China, Denmark, Japan, and Korea. To date, a subset of 356 accessions, primarily accessions from Russia, China and Japan, have been sent to USDA-APHIS-PPQ quarantine, and most of these have been released.

Field trials of the *M. sacchariflorus* accessions were established in spring 2015 at our location in IL, China, Denmark, Japan, Korea, and Saskatoon (Table 2). In addition to the *M. sacchariflorus* field trials, we also established at Illinois a replicated field trial of 321 *M. sacchariflorus* × *M. sinensis* (diploid × diploid) F₂s plus their parents and *M. xgiganteus* 'Illinois' as controls. Due to a combination of unusually wet weather during spring 2015 in Ontario and personnel changes at our industry collaborator in Leamington, ON, a field trial at this location was not established as had been originally planned. In place of the Leamington trial, in spring 2016 we established at Urbana, IL an additional field trial of segregating *M. sacchariflorus* × *M. sinensis* (diploid × diploid) F₁s based on two very high-yielding parents. For perennial grasses such as *Miscanthus*, the first year in the field is primarily for establishment, and years 2+ provide yield data. At the present time, we have observed strong overwintering ability and good spring regrowth at each of our hardiness zone 6 and warmer locations (Fig. 3). Encouragingly, we also observed winter-survival of some entries at our coldest location, Saskatoon. Though the northern hemisphere winter of 2015-2016 was unusually warm due to a strong El Nino, the lowest air temperature recorded during this winter in Saskatoon was -30 C, indicating that some of the *M. sacchariflorus* entries have exceptional tolerance to freezing temperatures during dormancy. Additionally, our collaborators in DK have observed that some of the *M. sacchariflorus* accessions originating from Russia had high rates of photosynthesis when air temperatures were <12 C, which enables them to grow earlier in the early spring when most other C4 grasses cannot grow or grow poorly. Further detailed studies of the most promising *M. sacchariflorus* accessions from Russia led to

the important discovery that some genotypes achieve rates of photosynthesis under chilling temperatures that are double that of the highly chilling tolerant cultivar *M. ×giganteus* 'Illinois'.

Population structure and identifying genes governing key traits:

Early in the project, we completed and published a study on the population structure of *M. sacchariflorus* from Russia. Subsequently, we publish the most comprehensive study to date on region-wide population structure of *M. sacchariflorus* from throughout the species' native range.

For the *M. sacchariflorus* accessions from Russia, which were previously collected by PI Sacks as part of USDA-funded plant collection expedition in 2012, the DNA was in-hand at Illinois at the start of the current project. The key results of this study were:

- 1) Nearly all the accessions from Russia were diploid, with only three tetraploid accessions out of 163. These rare tetraploid *M. sacchariflorus* accessions are adapted to USDA hardiness zone 3 and thus will be especially useful for breeding triploid *M. ×giganteus* that is strongly winter-hardy.
- 2) Genetic relatedness among the Russian accessions varied with geographic distance but no distinctly differentiated populations were identified.
- 3) We found no evidence for introgression of genes from *M. sinensis* into *M. sacchariflorus* for the Russian accessions, in contrast to our recently published study on gene flow between *M. sinensis* and *M. sacchariflorus* in Japan.
- 4) We observed great phenotypic variation among accessions at the collection sites for plant height, basal stem diameter, and number of stems per area. GWAS identified three significant QTL for number of stems per area, an important component of yield. If confirmed in our subsequent replicated multi-location field trials, this new approach will greatly facilitate germplasm use by providing plant breeders with important information, including candidate QTLs, about accessions.

For the region-wide study, we identified six groups of *M. sacchariflorus*, three diploid and three tetraploid (Fig. 2). Tetraploids were typically found in Japan, Korea and on the Shandong peninsula of China. Other key conclusions include:

1. During the last glacial maximum (LGM), an ancestral population of *M. sacchariflorus* existed in the region that is now the Yellow Sea, which was entirely land during the LGM. Three similarly-diverse populations are derived from the ancestral population: N China diploids, Korea diploids (later migrating to NE China and Russia), and Japan tetraploids.
2. Two independent tetraploidization events took place, one resulting in the Japanese populations and the other on the western edge of the Yellow Sea region (near or in N China), respectively. Western Yellow Sea basin tetraploids subsequently migrated to Korea.
3. Tetraploidy in *M. sacchariflorus* facilitates introgression from *M. sinensis* in Korea and S Japan where the flowering times of these two species overlap. Diploids in Korea and China exhibit hybridization without introgression.
4. *M. sacchariflorus* subsp. *lutarioriparius* is derived from N China diploid *M. sacchariflorus*, and exhibits a strong genetic bottleneck. Interestingly, S Japan *M. sacchariflorus* has some ancestry from *M. sacchariflorus* subsp. *lutarioriparius*.
5. Ornamental *M. sacchariflorus* available in North America comes from a very narrow genetic base. Diploid ornamentals originate from NE China, and tetraploids from S Japan.
6. Triploid *M. ×giganteus* hybrids occur naturally in both Korea and S Japan, but their agronomic performance may be expected to differ given that they have very different ancestries, both in terms of *M. sinensis* and *M. sacchariflorus*.

Phenotypic diversity and marker-trait associations:

Field trials of the diversity panel conducted at six locations over four years revealed great diversity among populations and individuals for key traits, such as biomass yield, plant height, stem diameter, and flowering time. At all trial sites, the tallest entries were *M. sacchariflorus* ssp. *lutarioriparius*, demonstrating the potential usefulness of this subspecies for breeding improved cultivars for high biomass yield. A manuscript documenting the diversity of traits and genotype by environment interactions is currently in preparation. An additional manuscript describing genome wide association analyses and genomic prediction models is also in preparation. A complementary genetic mapping study on the F2 interspecific population is also in preparation. Additionally, we developed and published near-infrared spectroscopy models to efficiently and rapidly analyze straw composition in *Miscanthus*.

Deliverables:Publications:

- 1) 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.
- 2) Clark, L.V., and E.J. Sacks. 2016. TagDigger: user-friendly extraction of read counts from GBS and RAD-seq data. *Source Code for Biology and Medicine* doi: 10.1186/s13029-016-0057-7.
- 3) Jin, X., X. Chen, L. Xiao, C. Shi, L. Chen, B. Yu, Z. Yi, J.H. Yoo, K. Heo, C.Y. Yu, T. Yamada, E.J. Sacks, and J. Peng. 2017. Application of visible and near-infrared spectroscopy to classification of *Miscanthus* species. *PLoS ONE* doi: 10.1371/journal.pone.0171360.
- 4) Jin, X., C. Shi, L. C.Y. Yu, T. Yamada, and E.J. Sacks. 2017. Determination of leaf water content by visible and near-infrared spectrometry and multivariate calibration in *Miscanthus*. *Frontiers in Plant Science* doi: 10.3389/fpls.2017.00721.
- 5) Jin, X., X. Chen, C. Shi, M. Li, Y. Guan, C.Y. Yu, T. Yamada, E.J. Sacks, and J. Peng. 2017. Determination of hemicellulose, cellulose and lignin content using visible and near infrared spectroscopy in *Miscanthus sinensis*. *Bioresource Technology* 241:603-609 2017/10 doi: <https://doi.org/10.1016/j.biortech.2017.05.047>.
- 6) Yoo, J.H., E.S. Seong, B.K. Ghimire, K. Heo., X. Jin, T. Yamada, L.V. Clark, E.J. Sacks, and C.Y. Yu. 2018. Establishment of *Miscanthus sinensis* with decreased lignin biosynthesis by *Agrobacterium*–mediated transformation using antisense *COMT* gene. *Plant Cell Tissue & Organ Culture* doi: <https://doi.org/10.1007/s11240-018-1389-6>.
- 7) Clifton-Brown, J., A. Harfouche, M.D. Casler, H.D. Jones, W.J. Macalpine, D. Murphy-Bokern, L.B. Smart, A. Adler, C. Ashman, D. Awty-Carroll, C. Bastien, S. Bopper, V. Botnari, M. Brancourt-Hulmel, Z. Chen, L.V. Clark, S. Cosentino, S. Dalton, C. Davey, O. Dolstra, I. Donnison, R. Flavell, J. Greef, S. Hanley, A. Hastings, M. Hertzberg, T. Hsu, L. Huang, A. Iurato, E. Jensen, X. Jin, U. Jørgensen, A. Kiesel, D. Kim, J. Liu, J.P. McCalmont, B.G. McMahon, M. Mos, P. Robson, E. J. Sacks, A. Sandu, G. Scalici, K. Schwarz, D. Scordia, R. Shafiei, I. Shield, G. Slavov, B.J. Stanton, K. Swaminathan, G. Taylor, A.F. Torres, L.M. Trindade, T. Tschaplinski, J. Tuskan, T. Yamada, C.Y. Yu, R. Zalesny, J. Zong, J. and I Lewandowski. 2018. Breeding progress and preparedness for mass-scale deployment of perennial lignocellulosic biomass crops switchgrass, miscanthus, willow, and poplar. *GCB Bioenergy* doi:10.1111/gcbb.12566.
- 8) Clark, L.V., X. Jin, K.K.Petersen, K.G. Anzoua, L. Bagmet, P. Chebukin, M. Deuter, E. Dzyubenko, N. Dzyubenko, K. Heo, D.A. Johnson, U. Jørgensen, J.B. Kjeldsen, H. Nagano, J. Peng, A. Sabitov, T. Yamada, J.H. Yoo, C.Y. Yu, S.P. Long, and E.J Sacks. 2018. Population structure of *Miscanthus sacchariflorus* reveals two major polyploidization events, tetraploid-mediated unidirectional introgression from diploid *M. sinensis*, and diversity centred around the Yellow Sea. *Annals of Botany* <https://doi.org/10.1093/aob/mcy161>.
- 9) Dong, H, S.V. Green, A. Nishiwaki, T. Yamada, J.R. Stewart, M. Deuter, and E.J Sacks. 2018. Winter hardiness of *Miscanthus* (I): Overwintering ability and yield of new *Miscanthus x giganteus* genotypes in Illinois and Arkansas. *GCB Bioenergy* doi:10.1111/gcbb.12588.
- 10) Charles P. Pignon, C.P., I. Spitz, E.J Sacks, U. Jørgensen, K. Kørup, and S.P. Long. 2019. Siberian *Miscanthus sacchariflorus* accessions surpass the exceptional chilling tolerance of the most widely cultivated clone of *Miscanthus x giganteus*. *GCB Bioenergy* doi: 10.1111/gcbb.12599.

Presentations:

- 1) Sacks, E.J. Genomics and breeding of *Miscanthus*. International Plant & Animal Genome XXIII Conference. San Diego, CA. 10-14 January 2015.
- 2) Sacks, E.J. *Miscanthus* breeding, genomics and evolution. Iowa State University, Department of Agronomy seminar series. 24 March 2015
- 3) Clark, L.V., and E.J. Sacks. TagDigger: user-friendly extraction of read counts from GBS and RAD-seq data. International Plant & Animal Genome XXIV Conference. San Diego, CA. 9-13 January 2016.
- 4) Sacks, E.J. Breeding of *Miscanthus* is aided by understanding its complex evolutionary history, including its relationship with *Saccharum*. McGill University, Department of Plant Science seminar series. 21 April 2016.
- 5) Sacks, E.J. *Miscanthus*: What we now know about its evolutionary history, its relationship to *Saccharum*, and opportunities/challenges for breeding. University of Illinois, PMPB seminar series. 30 November 2016.
- 6) Sacks, E.J. Quantifying Phenotypic and Genetic Diversity of *Miscanthus sacchariflorus* to Facilitate Knowledge of Directed Improvement of *M. ×giganteus* (*M. sinsensis* × *M. sacchariflorus*) and Sugarcane. 2017 DOE Genomic Sciences Program Annual Principal Investigator (PI) Meeting. Crystal City, VA. 5-8 February 2017.
- 7) Sacks, E.J. Population and quantitative genetics of *Miscanthus*, a biomass crop, an ornamental crop, and a genetic resource for improving sugarcane. HudsonAlpha Institute for Biotechnology seminar series. 12 April 2017.
- 8) Sacks, E.J. *Miscanthus* genetic resources for improving biomass yield and their potential to also improve sugarcane. Brazilian Bioenergy Science and Technology Conference – BBEST 2017 "Designing a Sustainable Bioeconomy". Campos do Jordão, SP, Brazil. 16-19 October 2017.
- 9) Sacks, E.J. Population and quantitative genetics of *Miscanthus*. Hokkaido University, Field Science Center for Northern Biosphere seminar series, sponsored by the Japanese Society of Plant Breeding and Crop Sciences in Hokkaido. 21 August 2018.

Training:

Trainee	Under-graduate	Graduate	Post-doctoral	Technical	Institution
Alfred Magehema				X	AU
Helle Baadsgaard				X	AU
Jens Bonderup Kjeldsen				X	AU
Fiorella Debora Briceño Nuñez		X			HU
Hironori Nagano				X	HU
Kossonou Guillaume Anzoua			X		HU
Maiko Ohta				X	HU
Michihiro Yamazaki	X				HU
Misako Hirono		X			HU
Sotaro Tsujii	X				HU
Tomoya Inoue	X				HU
Jae Hoo Choi		X			KNU
Ji Hye Yoo			X		KNU
Benjamin Baechle				X	UIUC
Eliot Lee				X	UIUC
Helen Gapsis				X	UIUC
Homayoun Watan				X	UIUC
Kayla Boparai				X	UIUC
Lindsay Clark			X		UIUC
Maertens, Colten				X	UIUC
Melina Salgado	X				UIUC
Myling Luu	X				UIUC
Jiying Chai		X			ZJU
Liang Zhu				X	ZJU
Mei Li				X	ZJU
Weijie Huang	X				ZJU
Xianyin Zhang				X	ZJU
Xiaolin Chen		X			ZJU
Yifei Jin				X	ZJU
Yuda Jin	X				ZJU
Zhuliang Su				X	ZJU
Count	7	5	3	16	31

Table 1. *M. sacchariflorus* entries included in the study and # released from USDA-APHIS-PPQ quarantine as of May 2016.

Country of Origin	Total	# in U.S.
China	341	131
Japan	169	55
Russia	165	162
South Korea	167	2
Unknown	6	6
Grand Total	848	356

Table2. Field trial sites.

Sites	# genotypes trialed	Lat	Long	Elev (m)	Hardiness Zone
Saskatoon, SK	50	52.2	-106.6	510	2
Sapporo, Japan	561	43.1	141.3	11	6
Urbana, IL	225	40.1	-88.2	131	6
Chuncheon, Korea	644	37.9	127.8	97	6-7
Foulum, Denmark	625	56.5	9.6	48	8
Zhuji, China	625	29.8	120.2	44	9

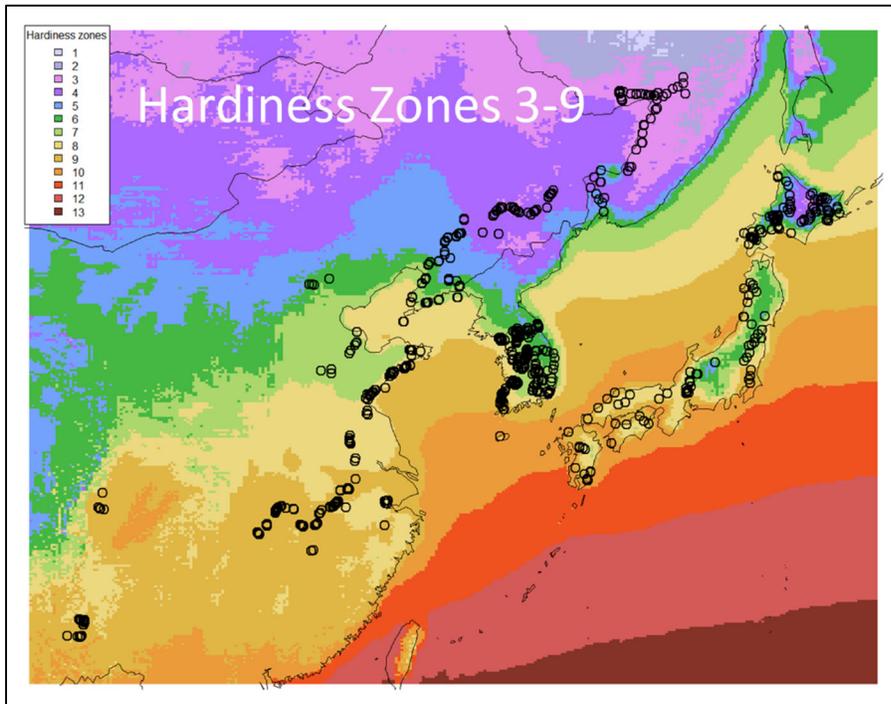


Figure 1. USDA hardiness zones at the collection sites of 847 *M. sacchariflorus* accessions.

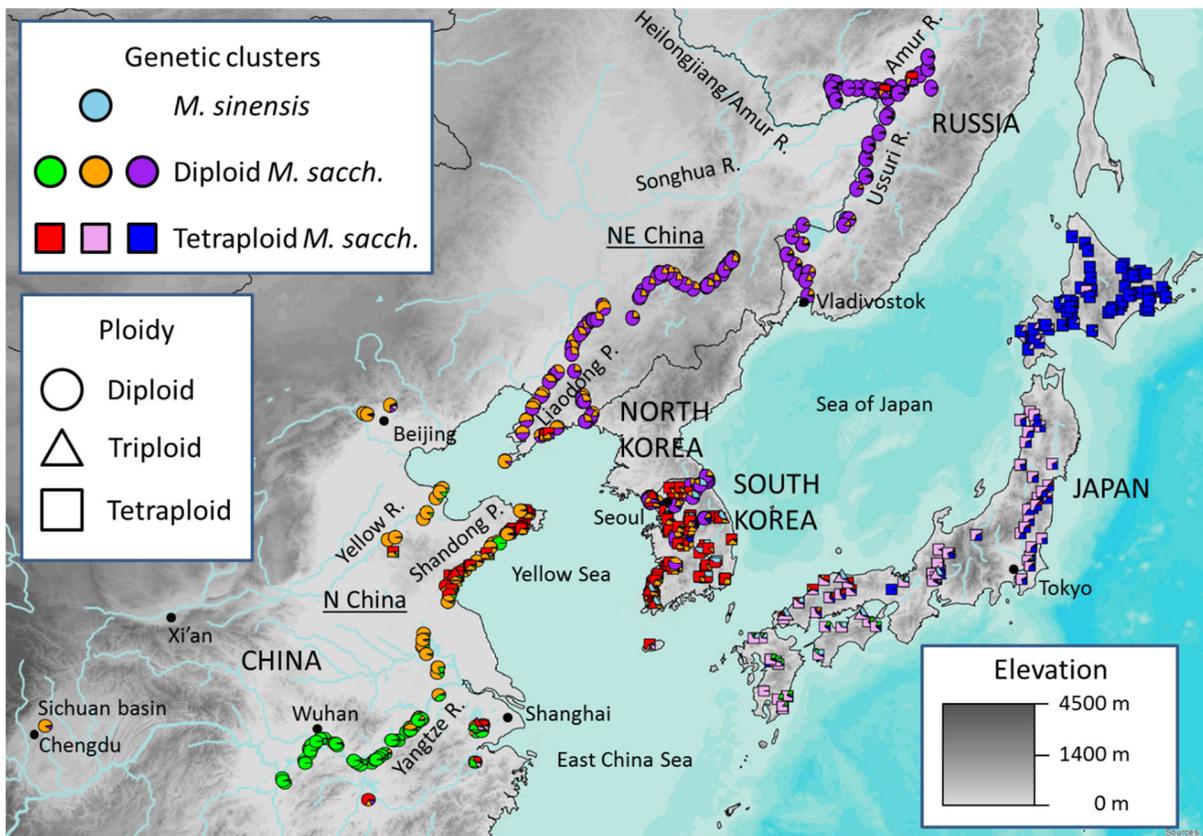


Figure 2. Population structure of *M. sacchariflorus*.



Figure 3. Great diversity among *M. sacchariflorus* accessions observed during May 2016 (year 2) at our field trials in Urbana, IL (top) and Zhuji, China (bottom).