

Supplementary Materials

**Assessment of drought tolerance of *Miscanthus* genotypes to use in
bioenergy crop development through dry-down treatment and fixed-soil-
moisture-content techniques**

Tzu-Ya Weng, Taiken Nakashima, Antonio Villanueva-Morales, J. Ryan Stewart, Erik J. Sacks⁵,

Toshihiko Yamada*

***Correspondence:**

Toshihiko Yamada

yamada@fsc.hokudai.ac.jp

SUPPLEMENTARY DATA

Supplementary Table S1, S2, S3, S4, &S5; Supplementary Figure S1 &S2

Supplemental Table S1. Detailed information of 29 *Miscanthus* genotypes used for the evaluation of low-water-adaptability capacity in *Miscanthus* spp., including entry number, species, origin location, and genetic groups background. Some information is not available for some genotypes

Species	Accession	Genetic clusters†	Type	Country of origin	Cultivar/	Place	Latitude	Longitude
Strain name								
<i>M. sacchariflorus</i>	UI11-00033	S Japan 4x Msa	Wild	Japan		Gifu		
<i>M. sacchariflorus</i>	JPN-2011-010	N Japan 4x Msa	Wild	Japan		Hokkaido	42.61618	141.8116
<i>M. sacchariflorus</i>	JPN-2011-004	S Japan 4x Msa	Wild	Japan		Gifu	36.16943	137.3095
<i>M. sacchariflorus</i>	JPN-2011-006	S Japan 4x Msa	Wild	Japan		Gifu	35.3246	136.693
<i>M. sacchariflorus</i>	JPN-2010-005	N Japan 4x Msa	Wild	Japan		Hokkaido	42.61475	141.8167
<i>M. sacchariflorus</i>	UI11-00031	Yangtze diploids (ssp. <i>lutarioriparius</i>) Msa	Wild	China				

<i>M. sacchariflorus</i>	RU2012-169	NEChina/Korea/Russia	Wild	Russia	Primorsky Krai	45.34596	133.5559
		diploids Msa					
<i>M. sacchariflorus</i>	RU2012-183	NEChina/Korea/Russia	Wild	Russia		43.75422	132.0818
		diploids Msa					
<i>M. sacchariflorus</i>	RU2012-056.1WD (4x)	NChina/Korea/Russia	Wild	Russia		48.82444	135.948
		tetraploids Msa					
<i>M. sacchariflorus</i>	JM11-006	S Japan 4x Msa	Wild	Japan	Yamaguchi	34.1986667	131.2806
<i>M. sacchariflorus</i>	UI10-00008	NEChina/Korea/Russia	Cultivar	Unknown	Hortico		
		diploids Msa					
<i>M. sacchariflorus</i>	RU2012-141	NEChina/Korea/Russia	Wild	Russia		47.50762	134.7411
		diploids Msa					

<i>M. sinensis</i>	PMS-164	Yangtze-Qinling Msi	Wild	China	Hebei	37.3400167	114.281
<i>M. sinensis</i>	PMS-007	Yangtze-Qinling Msi	Wild	China	Hubei	30.79765	110.2624
<i>M. sinensis</i>	UI10-00092	C Japan Msi	Cultivar				
<i>M. sinensis</i>	PMS-586	Sichuan Msi	Wild	China	Guizhou	27.0010333	108.699
<i>M. sinensis</i>	UI10-00024	S Japan Msi	Cultivar				
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	C Japan Msi	Cultivar				
<i>M. sinensis</i>	PMS-014	Sichuan Msi	Wild	China	Hubei	29.6570167	109.1195
<i>M. sinensis</i>	UI10-00097	S Japan Msi	Cultivar				
<i>M. sinensis</i>	UI10-00088	C Japan Msi	Cultivar				
					(Silver		
					Tower)		

<i>M. sinensis</i>	PMS-347	SE China Msi	Wild	China		Guangdong	24.1679833	115.8839
<i>M. sinensis</i>	UI10-00020	S Japan Msi	Cultivar			Adagio		
<i>M. sinensis</i>	PMS-285	Yangtze-Qinling Msi	Wild	China		Anhui	29.64345	118.1584
<i>M. sinensis</i>	UI10-00100	S Japan Msi	Cultivar			Yaku Jima		
<i>M. sinensis</i>	UI10-00053	S Japan Msi	Cultivar			Grosse		
						Fontaine		
<i>M. sinensis</i>	UI10-00080	C Japan Msi	Cultivar			Roland		
<i>M. sinensis</i>	UI10-00048	S Japan Msi	Cultivar			Gracillimus		
<i>M. floridulus</i>	PI417947	SE China Msi	Wild	Papua New Guinea		NG77-022		

†According to Clark *et al.* (2014) and Clark *et al.* (2019)

Supplemental Table S2-1. Least squares means of drought stress index (DSI) values of chlorophyll fluorescence (ϕ PSII) of *Miscanthus* genotypes in a screening experiment at Brigham Young University, Provo, Utah, USA

Species	Accession	Stress level†	DSI of ϕ PSII
<i>M. sinensis</i>	PMS-007	1	102.89 A‡
<i>M. sinensis</i>	UI10-00088	1	84.10 AB
<i>M. sinensis</i>	PMS-285	1	75.91 AB
<i>M. sinensis</i>	PMS-164	2	67.81 AB
<i>M. sacchariflorus</i>	UI11-00031	1	61.13 AB
<i>M. floridulus</i>	PI417947	1	59.20 AB
<i>M. sinensis</i>	PMS-347	1	52.71 AB
<i>M. sinensis</i>	PMS-014	1	52.58 AB
<i>M. floridulus</i>	PI417947	2	51.15 AB
<i>M. sinensis</i>	PMS-007	2	47.43 AB
<i>M. sinensis</i>	PMS-285	2	44.68 AB
<i>M. sinensis</i>	PMS-164	1	42.87 AB
<i>M. sacchariflorus</i>	UI11-00031	2	33.91 AB
<i>M. sinensis</i>	PMS-586	1	32.91 AB
<i>M. sinensis</i>	UI10-00088	2	16.86 AB
<i>M. sinensis</i>	PMS-014	2	0.019 AB

<i>M. sinensis</i>	PMS-586	2	-7.23 AB
<i>M. sinensis</i>	PMS-347	2	-17.19 B

†Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

‡Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S2-2. Least squares means of drought stress index (DSI) values of chlorophyll fluorescence (ϕ PSII) of *Miscanthus* genotypes in a precise-comparison experiment at Hokkaido University, Sapporo, Japan

Species	Accession	Stress level†	DSI of ϕ PSII
<i>M. sinensis</i>	PMS-285	2	163.24 A‡
<i>M. sinensis</i>	UI10-00024	2	114.06 AB
<i>M. sinensis</i>	PMS-164	2	98.95 ABC
<i>M. sinensis</i>	PMS-285	1	94.04 ABC
<i>M. sacchariflorus</i>	JPN-2011-004	2	80.80 ABC
<i>M. sinensis</i>	PMS-347	2	80.06 ABC
<i>M. sinensis</i>	UI10-00024	1	74.97 BC
<i>M. sinensis</i>	PMS-007	1	71.52 BC
<i>M. sinensis</i>	PMS-007	2	68.85 BC
<i>M. sacchariflorus</i>	UI10-00008	1	67.99 BC
<i>M. sinensis</i>	UI10-00020	2	65.80 BC
<i>M. sinensis</i>	PMS-164	1	63.64 BC
<i>M. sinensis</i>	UI10-00020	1	62.51 BC
<i>M. sacchariflorus</i>	UI11-00033	2	61.95 BC
<i>M. sacchariflorus</i>	UI10-00008	2	53.70 BC
<i>M. sacchariflorus</i>	JPN-2011-004	1	53.15 BC
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	2	51.87 BC

<i>M. sinensis</i>	PMS-347	1	47.97 BC
<i>M. sacchariflorus</i>	UI11-00033	1	39.22 C
<i>M. sinensis var. condensatus</i>	UI10-00015	1	37.81 C

†Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

‡Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S3-1. Least squares means of drought stress index (DSI) values of photosynthetic rate (Pn) of *Miscanthus* genotypes in a screening experiment at Brigham Young University, Provo, Utah, USA

Species	Accession	Stress level†	DSI of Pn
<i>M. sinensis</i>	PMS-164	2	124.48 A‡
<i>M. sinensis</i>	UI10-00088	1	99.62 A
<i>M. sinensis</i>	PMS-007	1	94.39 A
<i>M. floridulus</i>	PI417947	1	89.04 A
<i>M. sinensis</i>	PMS-285	1	86.75 A
<i>M. floridulus</i>	PI417947	2	82.02 A
<i>M. sinensis</i>	PMS-285	2	71.93 A
<i>M. sinensis</i>	PMS-007	2	66.02 A
<i>M. sacchariflorus</i>	UI11-00031	1	63.54 A
<i>M. sinensis</i>	PMS-014	1	56.38 A
<i>M. sinensis</i>	PMS-164	1	42.95 A
<i>M. sinensis</i>	PMS-586	1	39.28 A
<i>M. sinensis</i>	PMS-347	1	36.13 A
<i>M. sacchariflorus</i>	UI11-00031	2	35.86 A
<i>M. sinensis</i>	UI10-00088	2	21.59 A
<i>M. sinensis</i>	PMS-586	2	3.16 A
<i>M. sinensis</i>	PMS-014	2	-0.86 A

†Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

‡Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S3-2. Least squares means of drought stress index (DSI) values of photosynthetic rate (Pn) of *Miscanthus* genotypes in a precise-comparison experiment at Hokkaido University, Sapporo, Japan

Species	Accession	Stress level†	DSI of Pn
<i>M. sinensis</i>	PMS-007	1	77.31 A‡
<i>M. sacchariflorus</i>	UI11-00033	2	76.05 A
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	1	75.19 A
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	2	66.66 AB
<i>M. sacchariflorus</i>	JPN-2011-004	2	63.66 ABC
<i>M. sinensis</i>	UI10-00024	1	62.08 ABC
<i>M. sacchariflorus</i>	JPN-2011-004	1	61.20 ABC
<i>M. sinensis</i>	PMS-285	1	58.94 ABC
<i>M. sinensis</i>	PMS-007	2	56.27 ABC
<i>M. sacchariflorus</i>	UI11-00033	1	55.11 ABC
<i>M. sinensis</i>	PMS-285	2	54.60 ABC
<i>M. sinensis</i>	PMS-347	1	52.45 ABCD
<i>M. sinensis</i>	UI10-00020	1	51.97 ABCD
<i>M. sinensis</i>	PMS-164	1	37.64ABCD
<i>M. sinensis</i>	PMS-347	2	35.96 BCD
<i>M. sinensis</i>	PMS-164	2	34.95 CD
<i>M. sacchariflorus</i>	UI10-00008	2	15.46 D

† Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

‡ Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S4-1. Least squares means of drought stress index (DSI) values of stomatal conductance (gs) of *Miscanthus* genotypes in a screening experiment at Brigham Young University, Provo, Utah, USA

Species	Accession	Stress level†	DSI of gs
<i>M. sinensis</i>	PMS-164	2	223.67 A‡
<i>M. floridulus</i>	PI417947	1	205.71 A
<i>M. sinensis</i>	PMS-164	1	195.37 A
<i>M. sinensis</i>	UI10-00088	1	188.70 A
<i>M. sinensis</i>	PMS-007	1	181.12 A
<i>M. sinensis</i>	PMS-285	1	177.53 A
<i>M. sinensis</i>	PMS-007	2	117.85 A
<i>M. floridulus</i>	PI417947	2	109.87 A
<i>M. sinensis</i>	PMS-586	1	104.44 A
<i>M. sinensis</i>	PMS-285	2	96.88 A
<i>M. sinensis</i>	PMS-347	1	88.73 A
<i>M. sacchariflorus</i>	UI11-00031	1	79.86 A
<i>M. sinensis</i>	UI10-00088	2	53.99 A
<i>M. sinensis</i>	PMS-014	1	53.59 A
<i>M. sacchariflorus</i>	UI11-00031	2	31.96 A
<i>M. sinensis</i>	PMS-586	2	8.55 A
<i>M. sinensis</i>	PMS-014	2	-4.02 A

† Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

‡ Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S4-2. Least squares means of drought stress index (DSI) values of stomatal conductance (gs) of *Miscanthus* genotypes in a precise-comparison experiment at Hokkaido University, Sapporo, Japan

Species	Accession	Stress level†	DSI of gs
<i>M. sinensis</i>	PMS-164	1	824.29 A‡
<i>M. sinensis</i>	PMS-007	2	148.33 B
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	2	105.52 B
<i>M. sinensis</i>	UI10-00024	2	97.24 B
<i>M. sacchariflorus</i>	UI11-00033	1	70.15 BC
<i>M. sinensis</i>	PMS-285	2	43.53 BC
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	1	27.49 BC
<i>M. sinensis</i>	PMS-007	1	27.32 BC
<i>M. sinensis</i>	PMS-164	2	26.25 BC
<i>M. sinensis</i>	PMS-285	1	24.51 BC
<i>M. sinensis</i>	UI10-00024	1	24.14 BC
<i>M. sinensis</i>	PMS-347	1	15.97 BC
<i>M. sacchariflorus</i>	JPN-2011-004	1	9.21 BC
<i>M. sinensis</i>	UI10-00020	1	7.47 BC
<i>M. sacchariflorus</i>	UI10-00008	1	5.49 BC
<i>M. sacchariflorus</i>	JPN-2011-004	2	-16.64 BC
<i>M. sinensis</i>	PMS-347	2	-43.31 BC

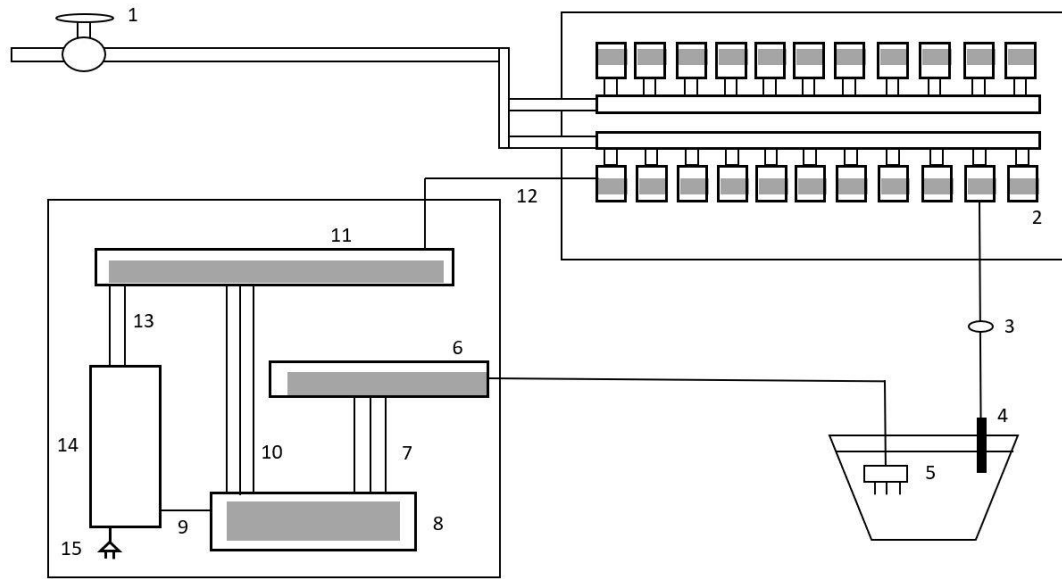
<i>M. sacchariflorus</i>	UI10-00008	2	-49.48 BC
<i>M. sinensis</i>	UI10-00020	2	-60.07 BC
<i>M. sacchariflorus</i>	UI11-00033	2	-118.04 C

†Stress level 1 represents the slight drought stress treatment and stress level 2 represents the severe drought stress treatment.

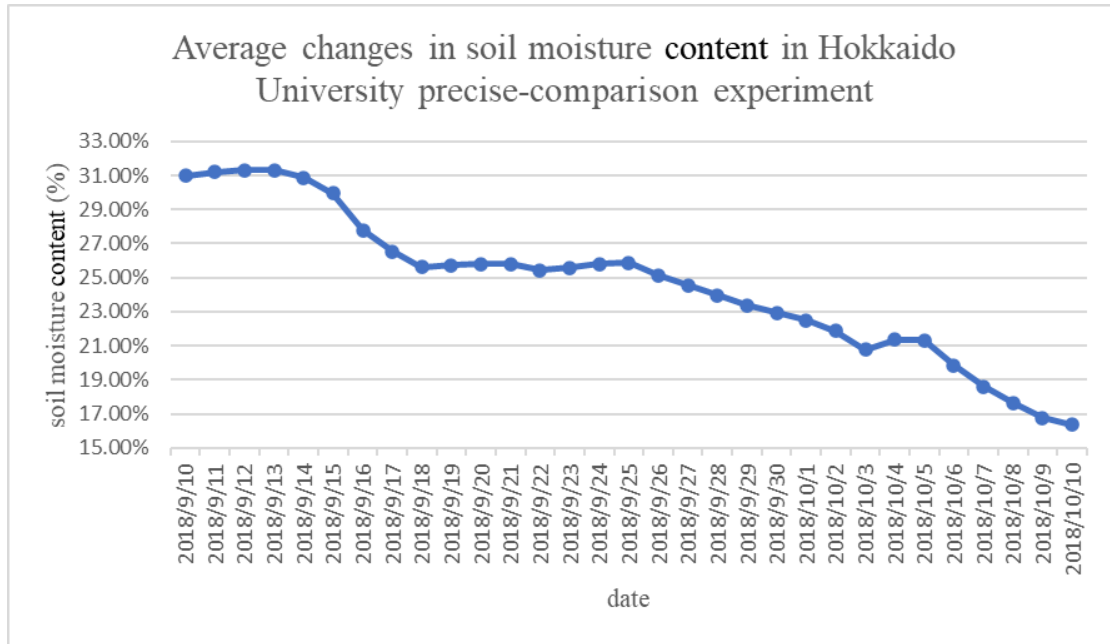
‡Least squares means with the same letter are not significantly different ($p < 0.05$).

Supplemental Table S5. Photosynthetic rate (Pn) of each *Miscanthus* genotype under each soil water content level in a precise-comparison experiment at Hokkaido University, Sapporo, Japan

Species	Accession	Pn ($\mu\text{mol CO}_2 \bullet \text{m}^{-2} \bullet \text{s}^{-1}$) in each soil water content level			
		30%	25%	20%	15%
<i>M. sinensis</i>	PMS-007	13.608	9.684	7.475	0.258
<i>M. sinensis</i>	PMS-285	15.008	7.761	7.457	-0.158
<i>M. sacchariflorus</i>	UI10-00008	13.844	1.160	2.281	0.308
<i>M. sinensis</i>	UI10-00020	24.656	12.798	11.650	2.947
<i>M. sinensis</i>	PMS-164	13.528	4.497	4.513	-1.812
<i>M. sinensis</i>	UI10-00024	17.117	10.304	9.341	1.526
<i>M. sinensis</i>	PMS-347	11.377	5.666	4.212	0.352
<i>M. sinensis</i> var. <i>condensatus</i>	UI10-00015	9.672	7.493	5.768	-0.054
<i>M. sacchariflorus</i>	JPN-2011-004	12.280	7.737	8.077	0.684
<i>M. sacchariflorus</i>	UI11-00033	13.692	7.433	10.404	0.681



Supplemental Figure S1 Simplified diagram showing various parts of the irrigation system, including the (1) water resource; (2) solenoid valve; (3) woodpecker emitter; (4) drip emitter; (5) soil moisture sensor; (6) thermocouple multiplexer; (7) connecting wires between CR6 datalogger and multiplexer; (8) CR6 datalogger; (9) power supply to CR6 datalogger; (10) connecting wires between CR6 datalogger and SDM 16 AC/DC relay controller; (11) SDM-CD16AC 16-Channel AC/DC relay controller; (12) power supply to solenoid valve; (13) power supply to SDM 16 AC/DC relay controller; (14) PS150 battery; and (15) main power supply. Only one pot is shown in detail (in the system, 30 pots can be independently irrigated).



Supplemental Figure S2 Average changes in soil moisture content controlled by the automated irrigation system in a precise-comparison experiment at Hokkaido University, Sapporo, Japan.