Nuclear SSR Markers for Miscanthus, Saccharum, and Related Grasses (Saccharinae, Poaceae)

Authors: Hodkinson, Trevor R., Cesare, Mariateresa de, and Barth, Susanne

Source: Applications in Plant Sciences, 1(11)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1300042

Applications in Plant Sciences 2013 1(11): 1300042

PRIMER NOTE

NUCLEAR SSR MARKERS FOR *MISCANTHUS*, *SACCHARUM*, AND RELATED GRASSES (SACCHARINAE, POACEAE)[1]

TREVOR R. HODKINSON[2,3,5], MARIATERESA DE CESARE[2,4], AND SUSANNE BARTH[4]

[2]School of Natural Sciences, Trinity College, Dublin D2, Ireland; [3]Trinity Centre for Biodiversity Research, Trinity College, Dublin D2, Ireland; and [4]Teagasc Crops Environment and Land Use Programme, Oak Park Research Centre, Carlow, Ireland

- *Premise of the study:* We developed nuclear simple sequence repeat (SSR) markers for the characterization of the biomass crop *Miscanthus*, especially *M. sacchariflorus*, *M. sinensis*, and *M. ×giganteus*, and tested for cross-species amplification.
- *Methods and Results:* Twenty-nine SSR markers (di- and tetranucleotide repeats) were developed from DNA sequences obtained from 192 clones from an enriched genomic library of *M. sinensis*. All markers were successfully amplified in *M. sacchariflorus*, *M. sinensis*, and *M. ×giganteus*, and 19 amplified across a broad range of *Miscanthus* species. Polymorphism information content and expected heterozygosity values (19 locus sample) were 0.88 and 0.89, respectively, for *M. sinensis*, 0.48 and 0.54 for *M. sacchariflorus*, and were the lowest in *M. ×giganteus* (0.33, 0.41). Thirteen out of 19 primer pairs showed cross-species amplification in non-*Miscanthus* sensu stricto taxa.
- *Conclusions:* The new set of 29 SSR markers will be of high value for characterizing *Miscanthus* germplasm collections, for prebreeding, and for assessing variation in natural populations.

Key words: cross-species amplification; microsatellites; *Miscanthus*; Poaceae; *Saccharum*; SSRs.

Miscanthus Andersson is under development as a biomass crop and has been characterized by a wide range of markers including amplified fragment length polymorphism (AFLP; Hodkinson et al., 2002), restriction fragment length polymorphism (RFLP; Hernández et al., 2001), inter-simple sequence repeat (ISSR) PCR, and DNA sequences of nuclear and chloroplast regions generated using conventional (Hodkinson et al., 2002) and next-generation approaches including RNAseq and genotyping by sequencing (GBS; Ma et al., 2012). Simple sequence repeat (SSR) markers from maize and *Brachypodium distachyon* (L.) P. Beauv. (Hernández et al., 2001; Zhao et al., 2011) have been successfully applied to *Miscanthus*, and chloroplast SSRs have been developed by De Cesare et al. (2010).

Some nuclear SSR markers have also been developed, such as those for *M. sinensis* Andersson, *M. floridulus* (Labill.) Warb. (Ho et al., 2011), and several other *Miscanthus* species (Zhou et al., 2011). However, there is a need to develop additional SSR markers for *Miscanthus* as the total number of available markers is limited. There is also a need to test these markers on a range of species, especially *M. sacchariflorus* (Maxim.) Hack., *M. sinensis*, and *M. ×giganteus* Greef & Deuter ex Hodk. & Renvoize as these comprise the main species of germplasm collections. SSRs developed from *Saccharum officinarum* L. expressed sequence tags (ESTs) have been recently used by Kim et al. (2012) to generate genetic maps of *M. sacchariflorus* and *M. sinensis* with genome coverage of 72.7% and 84.9%, respectively. The numbers of linkage groups found for the two maps (40 for *M. sacchariflorus* and 23 for *M. sinensis*) were higher than the basic chromosome number for *Miscanthus* ($x = 19$). Additional markers, such as those generated in this study, will be required to make more saturated maps, especially from noncoding regions that are underrepresented in current maps. Recently, single-nucleotide polymorphism (SNP) markers generated using GBS markers have been used for high-resolution mapping and identified all 19 linkage groups in *M. sinensis* (Ma et al., 2012).

METHODS AND RESULTS

DNA samples were either freshly extracted or obtained from the DNA bank at Trinity College, Dublin. Fresh leaves were frozen in liquid nitrogen and ground manually to a fine powder. Total genomic DNA was extracted following a modified cetyltrimethylammonium bromide (CTAB) method (Hodkinson et al., 2007). Total genomic DNA from the *M. sinensis* clone SW217 was used by ATG Genetics (Vancouver, British Columbia, Canada) to build a nuclear microsatellite–enriched library. After digestion with multiple 4-cutter restriction enzymes, enrichment for SSRs containing fragments was obtained through biotinylated TC_n, TG_n, and $GATA_n$ simple sequence motifs. The selected fragments were cloned into the EcoRI site of the plasmid pUC19 and screened for positive clones using ^{32}P-labeled TC_n, CA_n, and $GATA_n$ simple sequence motifs. Two 96-well microtiter plates containing single positive bacterial colonies, one selected for the presence of dinucleotide repeats and the second for the presence of tetranucleotide repeats, were produced. The 192 clones were sequenced by AGOWA GmbH (Berlin, Germany), and SSRs were identified in the clones using 'find microsat Win32' (Salamin, unpublished). All 192 clones contained SSRs (96 dinucleotides and 96 tetranucleotides). Eighty primer pairs were designed equally among these sets using Primer3 software (Rozen and Skaletsky, 2000; http://frodo.wi.mit.edu/primer3/) and tested with PCR. Selection of the final sample of 29 primers was based on clarity of product on an agarose gel. Primer details and GenBank numbers are provided in Table 1.

[1]Manuscript received 20 May 2013; revision accepted 12 July 2013.

The study was funded by the National Development Plan of Ireland through Teagasc core funding. M.d.C. was financed under the Teagasc Walsh Fellowship Ph.D. Scheme. We thank TCD Botanic Gardens Dublin; Svalöf Weibull, Sweden; Royal Botanic Gardens Kew, United Kingdom; and the University of Hohenheim, Germany, for sharing their *Miscanthus* resources.

[5]Author for correspondence: Trevor.Hodkinson@tcd.ie

doi:10.3732/apps.1300042

TABLE 1. Characteristics of 29 primer pairs developed for microsatellite genotyping.

Locus	Clone, GenBank accession no.	Repeat motif	Fluorescent dye	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	T_a (°C)	Sequence length (bp)	SSR size (bp)
Mis-1	SSR1A10, KF130838	$(TCTA)_{20}$	FAM	CAGTCCTTGGAGCAGGCTAT	AAGATCTCAAACCTATAGTC	54	202	80
Mis-13	SSR1F10, KF130839	$(TAGA)_{19}$	ROX	CGGACTAACTTGTGAATCTT	GTCCTTGGAGCAGGCTATGA	54	230	76
Mis-14	SSR1F12, KF130840	$(GATA)_{15}$	FAM	GTAGCTGCAACTGCTAGTGT	ACTCGCATTGGTTGGTATGA	59	141	60
Mis-15	SSR1F2, KF130841	$(ATCT)_{16}$	FAM	ACTACTGCATGCATCATGATG	TGCTTCGCGGCGAAGTTTCA	59	195	64
Mis-16	SSR1F5, KF130842	$(TATC)_{13}$/ $(TCTA)_{16}$	VIC	ATCTTGCCTAGGATGCATTAG	TGGTCTATTACAACAAGGCT	60	264	52+64*
Mis-20	SSR1G12, KF130843	$(TCTA)_{17}$	TAMRA	TAGCTGAGCTGTCTATGGTA	TAGCCATTGAGGCTAAGGAT	54	249	68
Mis-22	SSR1G8, KF130844	$(TAGA)_{17}$	VIC	CGAGCGAGCCTGCATGTGTG	TTGACGTCAGCAAGATATTG	54	173	68
Mis-23	SSR1G9, KF130845	$(ATCT)_{15}$	TAMRA	CACGAACTGAATCAGCATGC	GTAGCTGCAACTGCTAGTGT	60	240	60
Mis-24	SSR1H10, KF130846	$(AGAT)_{15}$	VIC	ATACACGATCCAAACATGTC	ATGTGCTCACCCAAGAGATG	60	324	60
Mis-33	SSR2B7, KF130847	$(CT)_{20}$	TAMRA	TGACATAGGGCTACACATAT	CGAGTGAGGCAGCTAGTTCA	48	242	40
Mis-37	SSR2D9, KF130848	$(TC)_{34}$	FAM	GAATGCAGTCATCAGCAGCT	TGGACATCTCTAGGTTGATC	54	218	68
Mis-41	SSR2F5, KF130849	$(GA)_{24}$	ROX	ATAATGCAGGTCAGTTCAAC	CGCAGCTAGCTGCTTGTCAG	54	226	48
Mis-42	SSR2F6, KF130850	$(AG)_{31}$	FAM	GCCGCCAGGCTCCCAAGCCT	ATCCGAGCCATGTATGCACG	54	206	62
Mis-50	SSR2H9, KF130851	$(GA)_{21}$	ROX	TACGGACGATTAACCAAGCC	CGCAAGGTGCAGGACCATCA	54	230	42
Mis-51	SSR2G4, KF130852	$(TC)_{20}$	FAM	GATCCATCACGGATTCATCA	ATCATAGGCAAAACGGATCG	60	164	40
Mis-52	SSR2C11, KF130853	$(GA)_{19}$	NED	TTATTGGTGCCCAAAGGTGT	AACAAGCCCTCAAGCTTCCT	60	370	38
Mis-53	SSR2G10, KF130854	$(GA)_{19}$	FAM	AGGCAGCACCTCACAAAACT	GGTGGAGATGCTCTTCTTGC	60	173	38
Mis-54	SSR2A11, KF130855	$(CT)_{18}$	NED	TAAGAAACGCAGCAGCAGAA	AGTCTCCGGCTTTCTCACAA	60	226	36
Mis-55	SSR2B9, KF130856	$(GA)_{18}$	VIC	CGGCTTCGAGTGATACCTTT	TACCGGATTTAAGGGGCTTT	60	250	36
Mis-59	SSR2B3, KF130857	$(GA)_{16}$	FAM	GAGCTGATCGCGTAGCAAG	TTCGATAAACAGGGGATTGG	60	152	32
Mis-60	SSR2C3, KF130858	$(GA)_{16}$	FAM	AGATGGCAGCTTGCTCTTGT	CCATTTGTTGAGCACGATGT	60	190	32
Mis-63	SSR1G3, KF130859	$(TCTA)_{14}$	VIC	AGGCTAGCACTTCCTCCAAA	CTGCCTGGTGACCCCTATAA	60	234	56
Mis-64	SSR1G6, KF130860	$(AGAT)_{14}$	NED	TCCCCTTAGTGTCCGTGAAG	GAGGCAGGTGTAGTCGGAGA	60	236	56
Mis-66	SSR1D5, KF130861	$(CTAT)_{13}$	VIC	CATGGCTACAGGCACCTAAAA	ATAACGAGAAATGGCCGATG	60	165	52
Mis-69	SSR1F4, KF130862	$(TCTA)_{13}$	NED	CCTCTGCGGATATGAGGTGT	GAAGTGACAACATGCGATGG	60	175	52
Mis-70	SSR1B10, KF130863	$(TATC)_{12}$	NED	TCGCACCTTTAATTTTTGCAT	TTATGAACCCGACAGGGAGA	60	249	48
Mis-71	SSR1D3, KF130864	$(TAGA)_{12}$	VIC	CAACCATGAGCACTTCTCCA	AACATAGGAGGCCAAGCAAA	60	179	48
Mis-78	SSR2G11, KF130865	$(CT)_{15}$	NED	TCTGCAGGTGACAAGGAAGA	GTCAACCGGCATAGTTCGAT	60	167	30
Mis-79	SSR2G9, KF130866	$(CT)_{15}$	VIC	GCCAACTCGTGGATTTGAGT	CGTAGCAAGAGGGGAACAAA	60	248	30

Note: T_a = annealing temperature.
* Compound SSR separated by a nonpolymorphic region.

Twenty-nine primer sets provided reliable amplification, and 19 of these were selected to have a mixture of di- and tetranucleotide SSRs. A template DNA volume of 1 µL (40 ng·µL^{-1}) was amplified with an initial denaturation of 5 min at 95°C followed by 35 cycles each with a denaturation of 1 min at 95°C, 1 min at a primer-specific annealing temperature (Table 1), and an extension of 1 min at 72°C, followed by a final extension at 72°C for 10 min. The reaction mixture (final volume) contained 1× reaction buffer containing 2 mM $MgSO_4$, 0.125 µM dNTPs, 0.25 µM of each primer, and 0.5 U of *Taq* DNA polymerase

TABLE 2. Genetic properties of the newly developed markers for three *Miscanthus* species.[a]

	M. sacchariflorus (n = 9)				*M. sinensis* (n = 73)				*M. ×giganteus* (n = 15)			
Locus	A	Size range (bp)	H_e	PIC	A	Size range (bp)	H_e	PIC	A	Size range (bp)	H_e	PIC
Mis-1	2	127–161	0.375	0.305	19	125–256	0.904	0.896	3	125–161	0.370	0.340
Mis-14	2	87–119	0.663	0.604	25	87–208	0.928	0.924	2	99–119	0.500	0.375
Mis-15	3	144–148	0.620	0.548	20	144–205	0.862	0.852	2	146–148	0.500	0.375
Mis-20	2	200–234	0.320	0.269	28	197–300	0.907	0.901	2	200–234	0.499	0.375
Mis-22	1	124	0.000	0.000	14	103–174	0.837	0.818	1	124	0.000	0.000
Mis-23	3	191–223	0.625	0.555	27	191–314	0.935	0.932	2	203–223	0.499	0.375
Mis-24	1	331	0.000	0.000	21	283–361	0.905	0.899	1	331	0.000	0.000
Mis-37	5	160–200	0.789	0.756	27	160–222	0.938	0.935	3	160–226	0.531	0.420
Mis-41	2	214–215	0.444	0.346	35	197–512	0.924	0.919	1	214	0.000	0.000
Mis-42	3	206–247	0.560	0.499	21	163–247	0.909	0.903	4	183–236	0.574	0.500
Mis-50	2	207–256	0.408	0.325	25	199–260	0.869	0.859	2	207–256	0.497	0.373
Mis-51	2	136–140	0.463	0.356	24	132–176	0.887	0.879	1	140	0.000	0.000
Mis-52	6	177–207	0.806	0.777	18	170–207	0.863	0.850	3	177–207	0.557	0.457
Mis-54	5	213–236	0.796	0.763	18	207–244	0.860	0.848	4	213–224	0.647	0.586
Mis-59	7	135–155	0.840	0.820	10	123–160	0.792	0.766	4	148–155	0.678	0.618
Mis-64	4	214–258	0.740	0.692	30	194–286	0.923	0.918	2	232–258	0.476	0.363
Mis-69	3	130–143	0.612	0.541	17	105–197	0.861	0.848	2	130–138	0.500	0.375
Mis-70	3	219–237	0.595	0.526	26	211–328	0.903	0.897	2	219–225	0.500	0.375
Mis-79	3	242–266	0.540	0.466	22	235–274	0.904	0.897	4	224–252	0.479	0.427
Mean			0.537	0.481			0.890	0.881			0.411	0.333

Note: A = number of alleles; H_e = expected heterozygosity; PIC = polymorphism information content.
[a] Statistics provided for species where sample size (n) was 9 or greater.

TABLE 3. Cross-amplification of the newly developed microsatellites of *Miscanthus*.[a]

			Saccharinae																					
			Miscanthus s.s.[b]						Miscanthus s.l.[c]						Other Saccharinae genera						Other Andropogoneae/Paniceae[d]			
Locus	*A* (n = 166)[e]	Size range (bp)[e]	*M. sacchariflorus*	*M. sinensis*	*M. sinensis* subsp. *condensatus*	*M. ×giganteus*	*M. transmorrisonensis*	*M. tinctorius*	*M. ecklonii*	*M. junceus*	*M. violaceus*	*M. nepalensis*	*M. nudipes*	*M. fuscus*	*Eulalia quadrinervis*	*Narenga porphyrocoma*	*Saccharum contortum*	*Saccharum officinarum*	*Saccharum spontaneum*	*Spodiopogon*[f]	*Sorghum halepense*	*Zea diploperennis*	*Cymbopogon citratus*	*Pennisetum* sp.
Mis-1	20	125–256	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-14	33	71–208	+	+	+	+	+	–	–	–	–	+	+	–	–	–	–	–	+	+	+	–	–	–
Mis-15	21	144–205	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Mis-20	33	197–300	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-22	16	103–174	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-23	30	176–314	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-24	23	248–361	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	+	–	+	–	–	–
Mis-37	33	169–226	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	+	–
Mis-41	44	131–512	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–
Mis-42	29	121–247	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
Mis-50	30	199–260	+	+	+	+	+	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–
Mis-51	27	132–176	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	–	–	–	–	–	–	–
Mis-52	22	132–207	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-54	20	207–244	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
Mis-59	13	123–162	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mis-64	40	177–286	+	+	+	+	+	+	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	–
Mis-69	24	105–220	+	+	+	+	+	+	–	+	+	+	–	–	–	–	+	+	–	–	–	–	–	–
Mis-70	31	211–328	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Mis-79	34	224–276	+	+	+	+	+	+	–	–	–	+	–	+	–	–	–	+	–	+	+	+	–	–
Average	27.5																							

[a] Cross-amplification in *Miscanthus* species, other Saccharinae, other Andropogoneae, and Paniceae (+ = yes; – = no).

[b] *Miscanthus* s.s. (Asian *Miscanthus* with basic chromosome number of 19).

[c] *Miscanthus* s.l. (GrassBase—The Online World Grass Flora [http://www.kew.org/data/grasses-db.html]).

[d] *Sorghum* is classified in Sorghinae (Andropogoneae), *Zea* in Tripsacinae (Andropogoneae), *Cymbopogon* in Andropogoninae (Andropogoneae), and *Pennisetum* in Cenchrinae (Paniceae).

[e] Total allele number and size range in base pairs (bp) for 19 nuclear SSR markers across all samples.

[f] *Spodiopogon rhizophorus* and *S. sibiricus.*

(New England BioLabs, Herts, United Kingdom). Five different fluorescent dyes were used for primer labeling to allow multiplexing, in pools (Table 1). A polyA treatment at 65°C was applied for 30 min to the PCR products. Undiluted PCR products were then sized using an ABI 3130xl automated DNA sequencer (Applied Biosystems, Carlsbad, California, USA) and the resulting peaks were scored with GeneMapper version 4.0 software (Applied Biosystems). All 29 primer pairs produced good amplification on eight test genotypes of *M. sacchariflorus*, *M. sinensis*, and *M.* ×*giganteus*, but 11 loci were not consistently amplified across our entire collection and were discarded from further analyses. Our final analysis therefore included 19 SSR markers. Allele number, size range, expected heterozygosity (H_e), and polymorphism information content (PIC) were calculated using PIC Calculator Extra (http://www.genomics.liv.ac.uk/animal/pic.html). H_e and PIC values were only calculated for *M. sacchariflorus*, *M. sinensis*, and *M.* ×*giganteus* because of sample size (Table 2).

Polymorphism at 19 microsatellite loci was studied in a collection of 166 individual grasses (Appendix 1), mostly belonging to the species *M. sinensis*, *M. sacchariflorus*, and *M.* ×*giganteus*. Fourteen individuals belonging to closely related genera were also included. All markers revealed considerable length polymorphism, with the number of alleles ranging from 13 to 44 per locus, with an average of 27.5 (Table 3). The loci amplified included a tetranucleotide repetition in nine cases and a dinucleotide repetition in the remaining 10. No major difference was observed between di- and tetranucleotide microsatellite loci in their ability to detect variation. Thirteen out of 19 primer pairs showed cross-amplification in non-*Miscanthus* species (Table 3). Average allele number was higher than the value of 12 found by Hernández et al. (2001) in a previous study using SSRs from maize. The higher number of clones used in our study (166 against 16 clones) and the introduction of species other than *M. sinensis*, *M. sacchariflorus*, and *M.* ×*giganteus* could account for the difference in allele number.

PIC and H_e values varied considerably among species (Table 2) and were the highest (0.88 and 0.89, respectively) for *M. sinensis*, 0.48 and 0.54 for *M. sacchariflorus*, and the lowest (0.33 and 0.41) in *M.* ×*giganteus*. The PIC value of *M. sinensis* (0.88) was consistent with the value of 0.83 in Hernández et al. (2001), both are higher than the average PIC value recently found by Zhao et al. (2011) in a study examining transferability of 49 microsatellite markers from *Brachypodium distachyon* to *M. sinensis*.

In the past few years, the first nuclear microsatellite markers for *Miscanthus* have been developed (Hung et al., 2009; Ho et al., 2011; Zhou et al., 2011). Both studies from Zhao et al. (2011) on transferability from *Brachypodium* P. Beauv. and from Hung et al. (2009) on nine new microsatellite loci specific for *Miscanthus*, were limited to *M. sinensis*, thus explaining the low level of polymorphism found compared to the markers in this study. Zhou et al. (2011) extended the test for their 14 newly developed markers to *M. floridulus*, *M. lutarioriparius* L. Liu ex S. L. Chen & Renvoize, and *M. sacchariflorus*, increasing the average number of alleles found to 16.1 and the PIC value to 0.76. A different approach was used by Ho et al. (2011) to develop 12 new SSR primer pairs for *Miscanthus*. They designed primers based on genic microsatellite loci (EST-SSRs) obtained through transcriptome sequencing and detected an average of 7.9 alleles per locus when tested on *M. floridulus* and *M. sinensis*.

CONCLUSIONS

The newly developed primers presented here were found to cross-amplify not only within *Miscanthus* species but also in other members of the Saccharinae, Andropogoneae, and Paniceae. They amplified DNA in *Zea* L. (Tripsacinae), *Sorghum* Moench (Sorghinae), *Cymbopogon* Spreng. (Andropogoninae), and *Pennisetum* Rich. (Paniceae). The primers are of high value for characterization of *Miscanthus* species and can be applied to other closely related genera including *Saccharum* L.

LITERATURE CITED

De Cesare, M., T. R. Hodkinson, and S. Barth. 2010. Chloroplast DNA markers (cpSSRs, SNPs) for *Miscanthus*, *Saccharum* and related grasses (Panicoideae, Poaceae). *Molecular Breeding* 26: 539–544.

Hernández, P., G. Dorado, D. A. Laurie, A. Martín, and J. W. Snape. 2001. Microsatellites and RFLP probes from maize are efficient sources of molecular markers for the biomass energy crop *Miscanthus*. *Theoretical and Applied Genetics* 102: 616–622.

Ho, C.-W., T.-H. Wu, T.-W. Hsu, J.-C. Huang, C.-C. Huang, and T.-Y. Chiang. 2011. Development of 12 genic microsatellite loci for a biofuel grass, *Miscanthus sinensis* (Poaceae). *American Journal of Botany* 98: e201–e203.

Hodkinson, T. R., M. W. Chase, C. Takahashi, I. J. Leitch, M. D. Bennett, and S. A. Renvoize. 2002. The use of DNA sequencing (ITS and *trnL-F*), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). *American Journal of Botany* 89: 279–286.

Hodkinson, T. R., S. Waldren, J. A. N. Parnell, C. T. Kelleher, K. Salamin, and N. Salamin. 2007. DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research* 120: 17–29.

Hung, K.-H., T.-Y. Chiang, C.-T. Chiu, T.-W. Hsu, and C.-W. Ho. 2009. Isolation and characterization of microsatellite loci from a potential biofuel plant *Miscanthus sinensis* (Poaceae). *Conservation Genetics* 10: 1377–1380.

Kim, C., D. Zhang, S. A. Aukland, L. K. Rainville, K. Jakob, B. Kronmiller, E. J. Sacks, et al. 2012. SSR-based genetic maps of *Miscanthus sinensis* and *M. sacchariflorus*, and their comparison to sorghum. *Theoretical and Applied Genetics* 124: 1325–1338.

Ma, X., E. Jensen, N. Alexandrov, and M. Troukhan. 2012. High resolution genetic mapping by genome sequencing reveals genome duplication and tetraploid genetic structure of the diploid *Miscanthus sinensis*. *PLoS ONE* 7: e33821. http://dx.plos.org/10.1371/journal.pone.0033821.

Rozen, S., and H. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Zhao, H., J. Yu, F. M. You, M. Luo, and J. Peng. 2011. Transferability of microsatellite markers from *Brachypodium distachyon* to *Miscanthus sinensis*, a potential biomass crop. *Journal of Integrative Plant Biology* 53: 232–245.

Zhou, H. F., S. S. Li, and S. Ge. 2011. Development of microsatellite markers for *Miscanthus sinensis* (Poaceae) and cross-amplification in other related species. *American Journal of Botany* 98: e195–e197.

Appendix 1. List of all accessions used in the study, source, and herbarium voucher number. All taxa are Andropogoneae subtribe Saccharinae unless indicated otherwise.

Taxon[a]	Source[b]	Voucher[c]
M. sacchariflorus 1	TCD Bot. Gardens	TCD P15
M. sinensis 'Zebrinus' 2	TCD Bot. Gardens	TCD P20
M. sinensis 'Zebrinus' 3	TCD Bot. Gardens	TCD P21
M. ×*giganteus* 4	TCD Bot. Gardens	TCD P34
M. ×*giganteus* 5	TCD Bot. Gardens	TCD P36
Miscanthus sp. 6	TCD Bot. Gardens	Tea-6
M. sinensis 7	TCD Bot. Gardens	TCD P48
Miscanthus sp. 8	TCD Bot. Gardens	TCD P50
M. sinensis 9	TCD Bot. Gardens	TCD P51
M. sacchariflorus 10	TCD Bot. Gardens	TCD P58
Miscanthus sp. 11	TCD Bot. Gardens	Tea-11
M. sinensis 13	TCD Bot. Gardens	TCD P73
M. sinensis 14	TCD Bot. Gardens	TCD P75
Miscanthus sp. 15	TCD Bot. Gardens	TCD P104
M. transmorrisonensis 16	TCD Bot. Gardens	TCD P105
M. ×*giganteus* 17	TCD Bot. Gardens	TCD P108
Miscanthus sp. 18	TCD Bot. Gardens	Tea-18
M. sinensis 'Goliath' 19	TCD Bot. Gardens	TCD P110, SIN-H6
M. ×*giganteus* 20	TCD Bot. Gardens	TCD P114
Miscanthus sp. 21	TCD Bot. Gardens	Tea-21
Miscanthus sp. 22	TCD Bot. Gardens	Tea-22
Miscanthus sp. 23	TCD Bot. Gardens	Tea-23
M. sinensis 24	TCD Bot. Gardens	TCD P11
M. sinensis 25	TCD Bot. Gardens	TCD P11
M. sinensis 26	TCD Bot. Gardens	TCD P11
Miscanthus sp. 27	TCD Bot. Gardens	Tea-27
Miscanthus sp. 28	TCD Bot. Gardens	Tea-28
Miscanthus sp. 29	TCD Bot. Gardens	Tea-29
M. sinensis 30	TCD Bot. Gardens	Tea-30
M. ×*giganteus* 31	TCD Bot. Gardens	Tea-31
M. ×*giganteus* 32	TCD Bot. Gardens	Tea-32
M. sinensis 'Zebrinus' 33	TCD Bot. Gardens	TCD P20
Miscanthus sp. 34	TCD Bot. Gardens	Tea-34
M. sinensis 'Gross Fontane' 35	TCD Bot. Gardens	TCD P30
M. sinensis 'Gross Fontane' 36	TCD Bot. Gardens	Tea-36
Miscanthus sp. 37	TCD Bot. Gardens	Tea-37
Miscanthus sp. 38	TCD Bot. Gardens	Tea-38
Miscanthus sp. 39	TCD Bot. Gardens	Tea-39
M. sinensis 40	TCD Bot. Gardens	TCD P62
Miscanthus sp. 42	TCD Bot. Gardens	Tea-42
Miscanthus sp. 43	TCD Bot. Gardens	Tea-43
M. sinensis subsp. *condensatus* 44	TCD Bot. Gardens	TCD P94
Miscanthus sp. 45	TCD Bot. Gardens	Tea-45
Miscanthus sp. 46	TCD Bot. Gardens	Tea-46
Miscanthus sp. 47	TCD Bot. Gardens	Tea-47
Miscanthus sp. 48	TCD Bot. Gardens	Tea-48
Miscanthus sp. 49	TCD Bot. Gardens	Tea-49
Miscanthus sp. 50	TCD Bot. Gardens	Tea-50
Miscanthus sp. 51	TCD Bot. Gardens	Tea-51
Miscanthus sp. 52	TCD Bot. Gardens	Tea-52
Miscanthus sp. 53	TCD Bot. Gardens	Tea-53
Miscanthus sp. 54	TCD Bot. Gardens	Tea-54
Miscanthus sp. 55	TCD Bot. Gardens	Tea-55
M. sinensis 'Goliath' 56	Teagasc Oak Park	Tea-56
M. sinensis 'Goliath' 57	TCD Bot. Gardens	Tea-57
M. sinensis 'Sirene' 58	Teagasc Oak Park	Tea-58
M. sinensis 'Strictus' 59	TRH garden	Tea-59
M. sinensis 'Strictus' 60	TCD Bot. Gardens	Tea-60
M. sinensis 'Malapartus' 61	TRH Garden	Tea-61
M. sinensis 62	TRH Garden	Tea-62
M. sinensis 'Sirene' 63	TCD Bot. Gardens	Tea-63
M. ×*giganteus* 64	TCD Bot. Gardens	Tea-64
M. ×*giganteus* 65	TCD Bot. Gardens	Tea-65
M. ×*giganteus* 66	TRH Garden	Tea-66
Miscanthus sp. 68	TCD Bot. Gardens	Tea-68
Miscanthus sp. 69	TCD Bot. Gardens	Tea-69
Miscanthus sp. 70	TCD Bot. Gardens	Tea-70
Miscanthus sp. 71	TCD Bot. Gardens	Tea-71

Appendix 1. Continued.

Taxon[a]	Source[b]	Voucher[c]
Miscanthus sp. 72	TCD Bot. Gardens	Tea-72
Miscanthus sp. 73	TCD Bot. Gardens	Tea-73
M. ×*giganteus* 74	Germany—from Denmark	Tea-M1 Lasei 1
M. sacchariflorus × *M. sinensis* 75	Germany	Tea-M81 RH 81
M. sinensis 76	Germany—from Japan	Tea-88-110
M. sinensis 77	Germany—from Japan	Tea-88-111
M. sinensis 78	Germany—from Japan	Tea-90-5
M. sinensis 79	Germany—from Japan	Tea-90-6
M. sinensis 80	Germany—from Sweden	Tea-SW 217
M. ×*giganteus* 81	Germany—from Denmark	Tea-M53 IPL 53
M. ×*giganteus* 82	Germany	Tea-M56 HAGA 56
M. ×*giganteus* 83	Germany	Tea-M63 GREIF 63
M. sacchariflorus 84	Germany—from Japan	Tea-M11 MATEREC 11
M. sinensis 'Goliath' 85	Germany	Tea-M7 GOFAL 7
M. sinensis hybrid 86	Germany	Tea-M42 BERBO 42
M. sacchariflorus × *M. sinensis* 87	Germany	Tea-M43RH43
M. sinensis hybrid 88	Germany	Tea-M78 JESEL 78
Miscanthus sp. 89	Oak Park	Tea-89
Miscanthus sp. 90	Oak Park	Tea-90
Miscanthus sp. 91	Oak Park	Tea-91
Miscanthus sp. 92	Oak Park	Tea-92
M. ×*giganteus* 93	IGER/TinPlant/Oak Park	Tea-93
M. ×*giganteus* 94	Old Trial Teagasc Oak Park	Tea-94
M. sinensis 95	Sweden	Tea-95
M. sinensis 96	Sweden	Tea-96
M. sinensis 97	Sweden	Tea-97
M. sinensis 98	Sweden	Tea-98
M. sinensis 99	Sweden	Tea-99
M. sinensis 100	Sweden	Tea-100
M. sinensis 101	Sweden	Tea-101
M. sinensis 102	Sweden	Tea-102
M. sinensis 103	Sweden	Tea-103
M. sinensis 104	Sweden	Tea-104
M. sinensis 105	Sweden	Tea-105
M. sinensis 106	Sweden	Tea-106
M. sinensis 107	Sweden	Tea-107
M. sinensis 108	Sweden	Tea-108
M. sinensis 109	Sweden	Tea-109
M. sinensis 110	Sweden	Tea-110
M. sinensis 111	Sweden	Tea-111
M. sinensis 112	Sweden	Tea-112
M. sinensis 113	Sweden	Tea-113
M. sinensis 114	Sweden	Tea-114
M. sinensis 115	Sweden	Tea-115
M. sacchariflorus × *M. sinensis* 116	Sweden	Tea-116
M. sacchariflorus × *M. sinensis* 117	Sweden	Tea-117
M. sacchariflorus × *M. sinensis* 118	Sweden	Tea-118
M. sacchariflorus × *M. sinensis* 119	Sweden	Tea-119
M. sacchariflorus × *M. sinensis* 120	Sweden	Tea-120
M. sacchariflorus × *M. sinensis* 121	Sweden	Tea-121
M. sacchariflorus × *M. sinensis* 122	Sweden	Tea-122
M. sacchariflorus × *M. sinensis* 123	Sweden	Tea-123
M. sacchariflorus × *M. sinensis* 124	Sweden	Tea-124
M. sacchariflorus × *M. sinensis* 125	Sweden	Tea-125
M. sacchariflorus × *M. sinensis* 126	Sweden	Tea-126
M. sacchariflorus × *M. sinensis* 127	Sweden	Tea-127
M. sacchariflorus 128	TCD Bot. Gardens	Tea-128
M. sacchariflorus 129	TCD Bot. Gardens	Tea-129
Miscanthus sp. 130	TCD Bot. Gardens	Tea-130
Miscanthus sp. 131	TCD Bot. Gardens	Tea-131
Saccharum officinarum	TCD Bot. Gardens	TCD TRH s.n.
Cymbopogon citratus[d]	TCD Bot. Gardens	TCD TRH s.n.
Zea diploperennis[e]	TCD Bot. Gardens	TCD TRH s.n.
Sorghum halepense 6[f]	RBG Kew 151 01	Kew 1966-54209
Pennisetum sp.[g]	TCD Bot. Gardens	TCD TRH s.n.
M. sinensis var. *variegatus* 1	RBG Kew 154 04	Kew 1969-19093
M. sinensis subsp. *condensatus* 7	RBG Kew 151	Kew 1969-19091
M. oligostachyus 16	RBG Kew 151 (pot)	Kew 1995-1864
M. nepalensis 25	RBG Kew TH 4	Kew 1985-8388

Appendix 1. Continued.

Taxon[a]	Source[b]	Voucher[c]
M. sinensis 'Goliath' 27	ADAS Steinmann nurseries	Kew MB93/02
M. sinensis 'Gracillimus' 28	ADAS Piccoplant, Germany	Kew MB94/05
M. sinensis 'Roland' 29	ADAS Piccoplant, Germany	Kew MB94/06
M. sinensis Anderss. 30	ADAS Wye College	Kew MB94/07
M. sinensis 'Gross Fontane' 31	ADAS Genft Dogels, Germany	Kew PN95/01
M. sacchariflorus 61	RBG Kew	Kew 1987-2727
M. sinensis 'Yakushimanum' 63	RBG Kew	Kew 1987-1148
M. transmorrisonensis 65	RBG Kew	Kew1990-2748
M. fuscus 82	RBG Kew	Kew 590
M. violaceus 84	RBG Kew	Kew 7437
M. ecklonii 86	RBG Kew	Kew 2347
M. junceus 88	RBG Kew	Kew 1060
M. junceus 89	RBG Kew	Kew 2309
M. ecklonii 105	RBG Kew	Kew 2929
M. ecklonii 106	RBG Kew	Kew 247
M. yunnanensis 107	RBG Kew	Kew 30689
M. nudipes 109	RBG Kew	Kew 2007
M. tinctorius 112	RBG Kew	Kew 1466
Saccharum spontaneum 117	RBG Kew	Kew Butt, 1977
Narenga porphyrocoma 120	RBG Kew	Kew 2092
Saccharum contortum 121	RBG Kew	Kew 3797
Spodiopogon rhizophorus 125	RBG Kew	Kew 283
Spodiopogon sibiricus 128	RBG Kew	Kew 210
Eulalia quadrinervis 134	RBG Kew	Kew 3294
M. sinensis 'Morning Light' 155	RBG Kew	Kew 1996 821
M. sacchariflorus 159	RBG Kew	Kew 3598 1935
M. sacchariflorus 160	RBG Kew	Kew 1984
M. tinctorius 'Nana Variegata' 161	RBG Kew	Kew 1996 1065
M. sinensis 'Goliath' 194	ADAS	Kew PN96/30

[a] Numbers accompanying species names represent the DNA extraction identifier for this study.

[b] Source abbreviations: ADAS = Agricultural Development Advisory Service (now Agriculture and Environmental Consultancy); IGER = Institute of Grassland and Environmental Research (now Institute of Biological, Environmental and Rural Sciences [IBERS]); RBG Kew = Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom; TCD Bot. Gardens = Trinity College Dublin Botanical Garden, Dublin, Ireland; Teagasc Oak Park = Teagasc Oak Park Research Centre, Carlow, Ireland; TRH Garden = personal garden of first author.

[c] Voucher abbreviations: Kew = Herbarium of the Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom; TCD = Trinity College Dublin Herbarium, Ireland; Tea = Teagasc Oak Park Research Centre, Carlow, Ireland.

[d] Andropogoninae, Andropogoneae (subtribe/tribe).

[e] Tripsacinae, Andropogoneae.

[f] Sorghinae, Andropogoneae.

[g] Cenchrinae, Paniceae.