NUCLEAR SSR MARKERS FOR Miscanthus, Saccharum, and Related Grasses (Saccharinae, Poaceae)¹

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• 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 Greffe & 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 TCn, TGn, and GATAn simple sequence motifs. The selected fragments were cloned into the EcoRI site of the plasmid pUC19 and screened for positive clones using 32P-labeled TCn, CA, and GATA 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 Skalický, 2000; http://primer3.sourceforge.net) 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.
## 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 | SSRA1A0, KF130838 (CTCA)$_{20}$ | FAM | TAMRA | CATGCTACGCTGCTATGTGTA | AACAGTTCAAAACCTAGTATC | 54 | 202 | 80 |
| Mis-13 | SSRIF10, KF130839 (TAGA)$_{19}$ | ROX | VIC | CGCACTACTTGGATAGCTTG | GTGCCCTGAGGGACGTGTA | 54 | 230 | 76 |
| Mis-14 | SSRIJ10, KF130840 (GATA)$_{16}$ | FAM | TAMRA | ATGCGTTACAGCTGCTATGTG | ATGCGTTACAGCTGCTATGTG | 54 | 191 | 60 |
| Mis-15 | SSRIJ2, KF130841 (ATCT)$_{16}$ | FAM | TAMRA | CTGCTACGCTGCTATGTG | CTGCTACGCTGCTATGTG | 54 | 195 | 64 |
| Mis-16 | SSRIJ5, KF130842 (TATC)$_{15}$ | VIC | TAMRA | ATGCTGTTACAGCTGCTATGTG | ATGCTGTTACAGCTGCTATGTG | 60 | 264 | 52+64* |

**Note:** $T_a$ is annealing temperature.

* Composite 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.*

| Locus | A | Size range (bp) | $H_a$ | PIC | A | Size range (bp) | $H_a$ | PIC | A | Size range (bp) | $H_a$ | PIC |
|-------|---|-----------------|------|-----|---|-----------------|------|-----|---|-----------------|------|-----|
| **M. sacchariflorus** (n = 9) | | | | | | | | | | | | |
| 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-23 | 3 | 124 | 0.000 | 0.000 | 14 | 103–174 | 0.837 | 0.818 | 1 | 124 | 0.000 | 0.000 |
| Mis-24 | 3 | 191–223 | 0.625 | 0.555 | 27 | 191–314 | 0.935 | 0.923 | 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 | 2 | 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 | 0.704 | 0.692 | 30 | 194–285 | 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 | 2 | 219–237 | 0.595 | 0.526 | 26 | 211–328 | 0.903 | 0.897 | 2 | 219–225 | 0.500 | 0.375 |
| Mis-79 | 2 | 242–266 | 0.540 | 0.466 | 22 | 235–274 | 0.904 | 0.890 | 4 | 224–252 | 0.479 | 0.427 |
| **M. sinensis** (n = 73) | | | | | | | | | | | | |
| **M. x giganteus** (n = 15) | | | | | | | | | | | | |

**Note:** $A$ = number of alleles; $H_a$ = expected heterozygosity; PIC = polymorphism information content.

*Statistically provided for species where sample size (n) was 9 or greater.

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### TABLE 3. Cross-amplification of the newly developed microsatellites of Miscanthus.a

| Locus | M. sacchariflorus | M. sinensis | M. sinensis subsp. condensatus | M. × giganteus | M. transmorisonensis | M. × ecklonii | M. × giganteus | M. × tenuiflorus | M. × violaceus | M. × nepalensis | M. × nudipes | M. × fuscus | Eulalia gigantea | Navelia panicea | Saccharinae species | Saccharum edule | Saccharum spontaneum | Spodiopogon rhizophorus | Sorghum halepense | Zea mays | Andropogon sp. | Cenchrus sp. |
|-------|-----------------|-------------|-------------------------------|----------------|---------------------|-------------|----------------|----------------|----------------|----------------|----------------|--------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Mis-1 | 20              | 125–256     | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| Mis-14| 33              | 71–208      | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| Mis-15| 21              | 44–205      | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| Mis-23| 30              | 136–314     | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| Mis-24| 23              | 248–361     | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| Mis-37| 33              | 189–226     | +                            | +              | +                   | +           | +              | +              | +              | +              | +              | +            | +              | +              | +              | +              | +              | +              | +              | +              | +              |
| 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| 275           |             |                              |                |                     |             |                |                |                |                |                |              |                |                |                |                |                |                |                |                |                |                |

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*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.
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M. lutarioriparius

Both studies from Zhao et al. (2011) on transferability from have been developed (Hung et al., 2009; Ho et al., 2011; Zhou et al., 2011). Brachypodium distachyon

of licate loci in their ability to detect variation. Thirteen out of 19 primer pairs

M. sinensis

polyA treatment at 65°C to cross-amplify not only within species but also

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 repeat 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. xiganteus 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. xiganteus. The PCR 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, M. lutaria riparius 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 (Sorghghinae), 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. Renoveize. 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. Chang, 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.

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.

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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                        | Source               | Voucher   |
|------------------------------|----------------------|-----------|
| *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 P31   |
| *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     | Tea-21    |
| Miscanthus sp. 21             | TCD Bot. Gardens     | Tea-22    |
| Miscanthus sp. 22             | TCD Bot. Gardens     | Tea-23    |
| Miscanthus sp. 23             | TCD Bot. Gardens     | Tea-29    |
| *M. sinensis* 24             | TCD Bot. Gardens     | Tea-30    |
| *M. sinensis* 25             | TCD Bot. Gardens     | Tea-31    |
| *M. sinensis* 26             | TCD Bot. Gardens     | Tea-32    |
| 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   | TCD Bot. Gardens     | 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  | TRH Garden           | Tea-59    |
| *M. sinensis* 'Malapartus' 61 | TRH Garden           | Tea-60    |
| *M. sinensis* 62             | TRH Garden           | Tea-61    |
| *M. sinensis* 'Sirene' 63    | TCD Bot. Gardens     | Tea-62    |
| *M. × giganteus* 64          | TCD Bot. Gardens     | Tea-63    |
| *M. × giganteus* 65          | TCD Bot. Gardens     | Tea-64    |
| *M. × giganteus* 66          | TRH Garden           | Tea-65    |
| Miscanthus sp. 68             | TCD Bot. Gardens     | Tea-66    |
| Miscanthus sp. 69             | TCD Bot. Gardens     | Tea-68    |
| Miscanthus sp. 70             | TCD Bot. Gardens     | Tea-69    |
| Miscanthus sp. 71             | TCD Bot. Gardens     | Tea-70    |
|                             |                      | Tea-71    |
| Taxon                      | Source                 | Voucher       |
|---------------------------|------------------------|---------------|
| Miscanthus sp. 72         | TCD Bot. Gardens       | Tea-72        |
| Miscanthus sp. 73         | TCD Bot. Gardens       | Tea-73        |
| M. × giganteus 74         | Germany                | Tea-M1 Lasei 1|
| M. sacchariflorus × M. sinensis 75 | Germany              | Tea-M81 RH 81 |
| M. sinensis 76            | Germany—from Denmark   | 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 Denmark   | Tea-SW 217    |
| M. × giganteus 81         | Germany                | 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   |
| 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 citratusa      | TCD Bot. Gardens       | TCD TRH s.n.  |
| Zea diploperennis         | TCD Bot. Gardens       | TCD TRH s.n.  |
| Sorghum halepense b       | RBG Kew 151 01         | Kew 1966-54209|
| Pennisetum sp. c         | RBG Kew 154 04         | Kew 1969-19093|
| M. sinensis var. variegatus 1 | RBG Kew 151          | Kew 1969-19091|
| M. sinensis subsp. condensatus 7 | RBG Kew 151 (pot)    | Kew 1995-1864 |
| M. oligostachyus 16       | RBG Kew TH 4           | Kew 1985-8388 |
| M. nepalensis 25          |                        |               |
### APPENDIX 1. Continued.

| Taxon                  | Source                      | Voucher         |
|------------------------|-----------------------------|-----------------|
| *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 Dgels, 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                    | Kew 1990-2748   |
| *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. ecklonii* 105        | RBG Kew                     | Kew 2309        |
| *M. ecklonii* 106        | RBG Kew                     | Kew 2929        |
| *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     |

* Numbers accompanying species names represent the DNA extraction identifier for this study.

<sup>a</sup> 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.

<sup>b</sup> Voucher abbreviations: Kew = Herbarium of the Royal Botanic Gardens, Kew, Richmond, Surrey, United Kingdom; Tea = Teagasc Oak Park Research Centre, Carlow, Ireland.

<sup>c</sup> Andropogoninae, Andropogoneae (subtribe/tribe).

<sup>d</sup> Tripsacinae, Andropogoneae.

<sup>e</sup> Cenchrinae, Paniceae.