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Genomics of the biomass crop *Miscanthus*: characterizing variation in the plastid genome and assessing nuclear ploidy variation



Key external stakeholders:

Miscanthus breeders, agronomists, plant science research community, general public

Practical implications for stakeholders:

Miscanthus is a genus of perennial C4 grasses that has raised interest as a non-food crop for energy and fibre production. Currently mostly one clone of *Miscanthus x giganteus* is used in most of the field trials. More genetic diversity is required to have accessions available suitable for different geographical areas.

- A field collection of ~150 *Miscanthus* genotypes has been established in Oak Park.
- The genetic diversity of a collection of 164 *Miscanthus* genotypes and 26 related species of the subfamily Panicoideae has been characterised using newly developed nuclear and chloroplast SSR markers.
- Morphological and agronomical characteristics of this field collection have been recorded.

Main results:

An Irish field collection of *Miscanthus* spp. genetic resources accessions was established in Oak Park. This field collection was characterized by morphology and by molecular means. Novel molecular markers have been developed for this purpose.

Opportunity / Benefit:

Energy crops, unlike food crops are grown largely to maximize their biomass without concern for the details of nutritional quality or flavor. The product is largely lingo-cellulosic material that is used to generate energy either directly by combustion or by conversion to other fuels such as bio-ethanol. Fossil fuels are becoming depleted and evidence is growing on the negative impacts of greenhouse gases on global climate. It is generally believed that alternative energy sources such as biomass, including *Miscanthus*, are required to facilitate a transition from non-renewable carbon resources to renewable sources. Advances in genetics/genomics are essential to breed the next generation of agroenergy crops. Since the *Miscanthus* enterprise is built around a very narrow genetic basis, more genetic diverse materials for certain geographical and environmental end uses.

Collaborating Institutions:

Trinity College Dublin

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1. Project background:

Miscanthus species are perennial, C4 grasses capable of tremendous biomass growth under a wide range of climatic conditions. Attention has focused on biomass crops because of the need for alternative energy sources for sustainable living. Very few Miscanthus genotypes have been assessed for their biomass potential but work is underway to develop new genotypes. The overall aim of this project is to gather essential genomic information required for plant breeding in this poorly understood genus. The focus will be on the characterization of the nuclear and cytoplasmic gene pools.

We will assess the cytoplasmic gene pools available for breeding by developing and applying molecular markers suitable for rapid screening of diversity. We will quantify diversity, group haplotypes and study infra-generic variation. We will also assess if cpDNA is maternally inherited in Miscanthus.

Miscanthus species form a polyploid complex including several other genera (including Saccharum). For polyploid complex assessment we will use flow cytometry. The aim is to study nuclear DNA content and ploidy variation in the breeding genepools and to compare these to the assessments of plastid genome variation. We will determine crossability groups and gather essential information for future ploidy manipulation.

2. Questions addressed by the project:

The projects aims to gather essential genomic information required for breeding in this poorly understood biomass crop. Cytoplasmic and nuclear gene pools will be assessed by developing molecular markers for the chloroplast and nuclear genomes. Ploidy variation data for the nuclear genome are being gathered and the morphological variability in the Oak Park field collection recorded.

- How large is the cytoplasmic and nuclear diversity in the Oak Park field collection?
- How variable the is the Oak Park field collection in its morphology?

3. The experimental studies:

A field collection of approximately 150 accessions was gathered from different sources and planted in two replications in the field in Oak Park with the aim to provide at least medium term storage of these genetic resources. The majority of this collection was donated by the botanic Gardens in Trinity College Dublin and by Svaloef Weibull/Sweden to provide a safety duplication of their collections. The collection was evaluated for morphological and agronomic characteristics. Reproductive morphological traits were recorded in detail to help in the identification of Miscanthus species. Nuclear microsatellite markers (nSSRs) and chloroplast microsatellite markers (cpSSRs) were developed from de novo and applied to the collection and further grass DNA samples. Genetic diversity for the chloroplast and nuclear genomes was calculated. Also the nuclear DNA contents of the accessions was determined using flow cytometry. Crossability studies were conducted in the greenhouse.

4. Main results:

Different traits were scored during the second growing season for the newly established collection of *Miscanthus* in Oak Park. A high level of variation was found for all the traits, with only a few of them showing a normal distribution in the multi-species dataset. When the same traits were measured in herbarium specimens from nine different species of *Miscanthus*, it was observed that mean values and standard deviation among species varied considerably, thus explaining the non-normal behaviour of a collection with mixed species. The ploidy level estimated in the collection ranged from diploid to tetraploid, with the genotypes almost equally divided between di- and triploid, with a few tetraploid. All the *M. sacchariflorus* were found to be tetraploid, and, as expected, the individuals classified as *M. xgiganteus* were triploid. Diploids were only recorded in *M. sinensis* and *M. condensatus*. The comparison between the ploidy levels and the data from the inflorescences showed that all the diploids that produced inflorescences had *sinensis*-like spikelets, while in the triploid group, the accessions identified as *M. sinensis* 'goliath' had *sinensis*-like spikelets, whereas among the *M. sacchariflorus* × *M. sinensis* hybrids, some carried *sinensis*-like inflorescences and some the *sacchariflorus*-like ones. None of the plants identified as *M. xgiganteus* or *M. sacchariflorus* flowered.

Starting from the complete sequence of the *Saccharum officinarum* chloroplast genome, a close ally to

Miscanthus, a total of 30 primer pairs were designed to amplify regions containing SSRs. With one exception, they all proved to be transferable to the genus *Miscanthus*. PCR products for the twelve primer pairs that performed better and amplified DNA reliably were sequenced to verify the presence in *Miscanthus* of microsatellite regions and possible polymorphism. Six markers showed length polymorphism of the repeats, with a species-specific preference in alleles. The six newly developed cpSSR primers were used to genotype the collection of *Miscanthus*. Their cross-amplification was also tested in closely related taxa. The results of the cpSSR genotyping revealed a high number of different haplotypes but with a clear bias in allele composition between *M. sinensis* and the two species *M. sacchariflorus* and *M. xgiganteus*, thus confirming *M. sacchariflorus* as the maternal lineage of the hybrid *M. xgiganteus*. The newly bred *M. sacchariflorus* × *M. sinensis* hybrids on the contrary shared their haplotype with *M. sinensis*.

New primer pairs for the amplification of nineteen nuclear SSRs loci were developed from the sequences of 192 clones from a microsatellite enriched library. The enrichment of the library was obtained by screening clones for sequences of TC_n, TG_n and GATA_n simple sequence motifs. The newly developed primers were used to characterize the genetic diversity in the *Miscanthus* collection and test their cross-amplification in closely related taxa. All nineteen markers showed high levels of polymorphism with an average number of alleles of 27.5 per locus.

5. Opportunity/Benefit:

The main objectives of this work were to develop new molecular markers for the genus *Miscanthus* and to characterize morphological and molecular diversity in a genetic resource collection of *Miscanthus* established in Oak Park, Carlow. Such characterization is essential pre-breeding work necessary to define gene pools, identify taxa, establish inter-relationships of the accessions and develop markers suitable for association studies, quantitative trait loci mapping and marker-aided selection.

6. Dissemination:

The project resulted in scientific publications and presentations at meetings. The project was also presented to visitor groups and at Open Days in Oak Park.

Main publications:

Hodkinson T.R.H., de Cesare M., Prickett R., Jones M.B. and Barth S. (2012) Genetic variation in *Miscanthus x giganteus* (Poaceae) a bioenergy and fibre crop. Irish Plant Scientists' Association Meeting 3rd to 5th April 2012, National Botanic Gardens Dublin.

de Cesare M., Hodkinson T.R. and Barth S. (2010) 'Chloroplast DNA markers (cpSSRs, SNPs) for *Miscanthus*, *Saccharum* and related grasses (Panicoideae, Poaceae)' *Molecular Breeding* 26: 539-544 (DOI: 10.1007/s11032-010-9451-z).

de Cesare M., Hodkinson T.R., Barth S. (2010) Development and use of nuclear microsatellite markers (SSRs) for genetic diversity evaluation in *Miscanthus* (Panicoideae, Poaceae). Irish Plant Scientists' Association Meeting 2nd to 4th June 2010, UCD Dublin.

Barth S. (2009) Genetic improvement of bioenergy crops (ed. Wilfried Vermerris) book review by S. Barth. Springer. *Annals of Botany* 103: viii-ix.

de Cesare M., Hodkinson T.R., Barth S. (2009) Phylogenetic analyses and ploidy variation in the biomass crop *Miscanthus*. Agricultural Research Forum 2009, Tullamore, p. 125 (ISBN 1-84170-538-1).

de Cesare M., Hodkinson T.R. and Barth S. (2009) Development of cpSSR markers in *Miscanthus* (Poaceae) Irish Plant Scientists' Association Meeting 19-20 March 2009, Trinity College Dublin.

de Cesare M., Hodkinson T.R. and Barth S. (2008) Use of cpSSRs markers and phylogenetic analyses in genus *Miscanthus*. ABIC conference, Cork August 2008, Ireland.

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