EVALUATION OF MICROSATELLITES (SIMPLE SEQUENCE REPEATS) AS GENETIC MARKERS IN SUGARCANE

BY

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Abstract

Cultivated sugarcane genotypes (Saccharum spp.) are derived from complex interspecific hybridisations between the species S. spontaneum and S. officinarum. To analyse this complex genome, we have investigated the potential of sugarcane microsatellite sequences as genetic markers in terms of their abundance, variability and ability to detect polymorphisms. From an enriched sugarcane genomic DNA microsatellite library, primer sets were designed and synthesised for over 200 microsatellite sequences. These were tested over five Saccharum genotypes. The number of alleles recorded per marker ranged between 2 and 12 (mean of 8). Markers that showed polymorphism had a Polymorphism Information Content (or PIC, where a value of 0 = monomorphic and 1 = highly polymorphic) value between 0.48 and 0.8 (mean of 0.72). Fluorescently labelled primers for eight of these microsatellite markers were tested on a population of 20 sugarcane cultivars. Results indicated the ability of these markers to accurately fingerprint genotypes, predict relationships, be used in genetic mapping and assist in selecting specific, genetically diverse parents for use in introgression. However, these genomic DNA derived microsatellites transferred poorly to related genera. By comparison, sugarcane microsatellites derived from EST sequences had a low PIC level when tested within cultivars, but a significantly higher level of polymorphism when applied to progenitor and related species.

Characteristics of SSRs from different sources

As part of an international collaborative effort, an enriched SSR library (i.e. of anonymous SSRs) was created from sugarcane genomic DNA (Edwards et al., 1996; Cordeiro et al., 1999, 2000). This library was characterised and compared with sugarcane SSRs mined from a sugarcane EST library (Cordeiro et al., 2001).

The frequency of non-redundant SSRs of seven or more dinucleotide repeats and five or more trinucleotide repeats in the total population of cDNA clones in the EST database was 2.88% compared with 57.3% (Cordeiro et al., 2000) from enriched sugarcane genomic DNA libraries. Differences in the frequency of repeat unit lengths and motif types are compared in Table 1.

The ability of the SSRs from the two sources to detect polymorphisms differed. Of 35 primer sets designed and synthesised for EST SSRs, 21 amplified products in both sugarcane cultivars and ancestral Saccharum species. Seventeen were polymorphic in two or more of five Saccharum genotypes tested, representing 6.8% of total EST SSRs isolated (Cordeiro et al., 2001). With anonymous SSRs, 254 primer pairs were designed and synthesised. 183 were polymorphic, representing 20% of total SSRs isolated (Cordeiro et al., 2000). Relatively few alleles were detected by the EST SSRs within five Saccharum genotypes tested [cv. Q124 from which the library was constructed (as a positive size control); cv. Q117 (both developed by the Bureau of Sugar Experiment Stations, Australia); cv. R570 (developed at the Centre d’Essai, de Recherche et de Formation (CERF), Réunion; and the ancestral species S. officinarum var. Chittan and S. spontaneum var. Saigon]. Allele numbers ranged from 0 to 5.0, with an average of 2.7. Anonymous SSRs detect a greater number of alleles, averaging 8.0 (Cordeiro et al., 2000). When the analysis was extended to related genera, the average number of alleles detected by EST SSRs increased to 7.5 while that for anonymous SSRs fell to 3.0.

Cross transferability

Of the polymorphic EST SSRs, five were randomly selected and found to be cross-transferable to the related genera, Sorghum and Erianthus (Figure 1). This is in contrast to anonymous SSRs where only 3 of 20 primer pairs tested transferred to closely related genera. The mean PIC value of EST SSRs tested on cultivated Saccharum cultivars was 0.23. With anonymous SSRs, the mean value was 0.72 (Cordeiro et al., 2000). However, the mean PIC value of EST SSRs increased to 0.62 when transferred to sugarcane ancestral species (S. officinarum and S. spontaneum) and to 0.80 when transferred across to closely related genera (Erianthus and Sorghum spp.). Conversely, the mean PIC value of anonymous SSR primers decreased to 0.66 when transferred to sugarcane ancestral species and to 0.00 when transferred to closely related genera. This is despite the fact that the same primer pairs have a PIC value of >0.5 when tested over the 5 Saccharum genotypes. These differences in the level of polymorphism within the

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Table 1—Characteristics of SSRs from sugarcane ESTs compared to SSRs derived from enriched sugarcane genomic DNA libraries (Cordeiro et al., 2001).

<table>
<thead>
<tr>
<th></th>
<th>EST SSRs</th>
<th>Genomic SSRs</th>
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<tbody>
<tr>
<td>Total SSRs characterised</td>
<td>250</td>
<td>1165</td>
</tr>
<tr>
<td>Frequency in library (%)</td>
<td>2.9</td>
<td>53.5</td>
</tr>
<tr>
<td>Ave. repeat length</td>
<td>6.1</td>
<td>13.7 (12.0†)</td>
</tr>
<tr>
<td>Ave. no. alleles detected when tested across 5 Saccharum genotypes</td>
<td>2.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Ave. no. alleles detected when tested across related genera</td>
<td>7.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Types of repeats (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>By nucleotide string</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinucleotides</td>
<td>9.7</td>
<td>46.2</td>
</tr>
<tr>
<td>Trinucleotides</td>
<td>90.3</td>
<td>30.6</td>
</tr>
<tr>
<td>Other†</td>
<td>0.0</td>
<td>23.2</td>
</tr>
<tr>
<td>By form*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfect</td>
<td>100.0</td>
<td>73.3</td>
</tr>
<tr>
<td>Imperfect</td>
<td>0.0</td>
<td>32.3</td>
</tr>
<tr>
<td>Compound</td>
<td>7.3</td>
<td>24.9</td>
</tr>
</tbody>
</table>

† For Trinucleotide repeats
‡ Mono, Tetra and Penta-nucleotides
§ Not searched for
* Compound repeats include combinations of perfect and imperfect repeats, which are already included in the relevant categories.

Saccharum genus and across genera between the two SSR sources are graphically depicted in Figure 1.

**Fingerprinting**

The difference in PIC values of SSRs derived from the two sources indicates that fingerprinting of sugarcane cultivars is best carried out using SSRs derived from genomic DNA. Markers from this source have proven to be robust in their ability to consistently and correctly identify individual clones. This has been seen in results produced by two separate laboratories using four anonymous SSRs tested over 20 sugarcane cultivars. Identical fingerprints were obtained allowing the unambiguous identification of the individuals sampled. In addition, these authors have shown that, with the use of phylogenetic
software programs, data derived from fingerprinting has the potential to determine relationships amongst cultivars and related species.

**Mapping**

In a trial to determine the suitability of SSRs for mapping in sugarcane, 22 genomic DNA derived SSR markers were used as RFLP probes to analyse 81 progeny of an Australian mapping cross (Q117 × 74C42). Of these, 19 segregated in a 1:1 pattern indicating that these markers should be readily mappable. In addition, 31 SSR markers were found to be polymorphic in at least one sorghum cross and one sugarcane cross. Such SSRs can be mapped in sorghum and sugarcane and could provide valuable anchor points in comparative analysis between the two genomes (McIntyre, C. L., pers. comm.).

**Discussion and conclusions**

Polymorphic EST SSRs comprised just 0.2% of total ESTs mined (Cordeiro et al., 2001), while polymorphic anonymous SSRs from enriched libraries was 12.5% (Cordeiro et al., 2000). Both these figures are comparable with the average success rate found in species such as tea-tree, sorghum, grape and wheat (Scott et al., 2000; Rossetto et al., 1999; Brown et al., 1996; Bryan et al., 1997; Roder et al., 1995).

However, the disparity in the absolute number of polymorphic primers from both sources makes comparison between the two groups difficult. Attempts were, however, made to maintain this perspective when comparing characteristics of SSRs derived from the two sources.

From our studies, sugarcane genomic DNA derived SSRs appear to be suited to fingerprinting of cultivars and determining diversity within these cultivars and progenitor species. EST SSRs are, however, suited to determining diversity between cultivars and closely related genera. The poor genetic diversity and lack of recent genetic input into cultivated cane has been an area of concern, and breeders have looked to related genera such as Erianthus for introgression of novel genetic material into the germplasm (Walker, 1987; Daniels and Roach 1987; Harvey et al., 1998). The use of EST-derived SSR markers will allow the characterisation of the genetic variability available in germplasm collections of closely related genera suitable for introgression into cultivated sugarcane. The difficulty of identifying true Saccharum × Erianthus hybrids based on morphological traits, previously a limiting factor in the successful introgression of the Erianthus genome into sugarcane, may also be assisted through the use of EST SSRs as an analytical tool.

**REFERENCES**


