UTILITY OF SSRs FOR DETERMINING GENETIC SIMILARITIES AND RELATIONSHIPS IN SACCHARUM AND ITS RELATED GENERA

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Abstract

Simple sequence repeats (SSRs), when resolved using agarose gels, may be a viable and cost-effective alternative to RFLPs and isozymes. Genotypes of Saccharum and its related genera were assayed for polymorphisms at 100 SSR marker loci. The alleles identified provide data for estimating genetic similarity among the genotypes. Of the 100 primer pairs, 32 pairs produced distinguishable and polymorphic bands. The pattern of genetic divergence revealed by the SSR polymorphisms of 32 primer pairs was consistent with known pedigrees. The utility of polymerase chain reaction (PCR)-based markers such as SSRs for measuring genetic similarities and relationships equals or exceeds that of RFLP markers, a property that may prove a valuable asset for sugarcane breeding programs.

Introduction

The improvement of any crop species is facilitated by introducing novel genes for traits such as yield, pest resistance and abiotic stress tolerance. One option is via the introgression of genes from related genera. These primers are mostly 16- to 17-mers. The level of polymorphism within the plant species studied thus far has been greater than that found by RFLPs (Nagaoka and Ogihara, 1997). There are several efforts underway to utilise SSR markers to study genetic diversity within germplasm collections and to characterise population dynamics (Wu and Tanksley, 1993; Senior and Heun, 1993). Here, research to evaluate the utility of SSRs to determine genetic similarities and relationships in Saccharum and related genera is presented.

Materials and methods

A total of 100 SSR primers from University of British Columbia Biotechnology Lab (UBCBL) primer set UBC#9 were tested for PCR amplification using a sample set of 11 genotypes including individuals of S. officinarum, S. sinense, S. barberi, S. robustum, S. spontaneum and its wild relatives, Erianthus rockii, Sorghum arundinaceus and Miscanthus floridulus.

These primers are mostly 16- to 17-mers. The PCR reactions were performed in 96-well microtitre plates using a Perkin Elmer 9600 thermocycler. The profile began with an initial denaturation step at 94°C for 7 min followed by 45 cycles at 94°C for 30 s, 52°C for 45 s, and 72°C for 2 min. Samples were held at 94°C for 7 min upon completion of cycling, then stored at 4°C until electrophoresis.

Results and discussion

Amplification products were resolved on 1.5% agarose gels, and the banding patterns were compared among the eleven genotypes. Of the 100 primer pairs tested, 68 pairs failed to produce clear, easily scorable bands. The remaining 32 pairs generated a total of 410 polymorphic bands across all the tested genotypes. Typical band patterns on 1.5% agarose gels are presented in Figure 1A.

Primer pairs, which produced distinct bands included 19 pairs selected for amplification of dinucleotide repeats, three pairs for trinucleotide repeats, two for tetranucleotide repeats and three for pentanucleotide repeats. Four primers selected for 5 degenerate motifs also yielded discrete bands. Primers based on (AC)n repeats produced the highest level of polymorphism, followed by 5 degenerate motifs and (GT)n. All primers designed to (AG)n, (AG)n or (TC)n is the most abundant dinucleotide repeat. Four hundred and ten polymorphic bands were detected in the genotypes. PCR amplification using ISSR (inter-simple sequence repeat) sequences as the primer produced 6.6 bands, on average, for species in the genus Saccharum, and 11.6 bands for related genera. The five species of Saccharum shared a total of 280 bands.

KEYWORDS: Sugarcane, Microsatellites, SSRs, Saccharum, Polymorphism.
Based on the distinguishable banding patterns obtained using 32 primers, a similarity matrix was generated using the similarity coefficient of Nei and Li (1979). A dendrogram was constructed using Vilber Lourmat gel analysis software. Cluster analyses of SSRs indicate significant genetic differences between *Saccharum* and its relatives, *Sorghum arundinaceus*, *Erianthus rockii* and *Miscanthus floridulus* (Labill).

The dendrogram also shows that *Saccharum* can be broken into two distinct groups. The first group is composed of one clone of *S. officinarum*, one of *S. barberi*, three from *S. robustum* and two clones from *S. sinense*. Another group is composed solely of one clone of *S. spontaneum* (Figure 1B).

A further 15 *S. spontaneum* clones were profiled with a subset of 20 primer pairs (data not shown). The results showed that *S. spontaneum* appears to be more diverse than the other species tested. This conclusion will require further experimental data to confirm.

**Conclusions**

Microsatellite marker studies suggest that the autopolyploid, *S. spontaneum*, differs from the other *Saccharum* species and from hybrids containing contributions from *S. spontaneum*. The other wild species, *S. robustum*, together with the cultivated races *S. officinarum*, *S. barberi* and *S. sinense*, belong together in one group to which nomenclatural priority may assign the name *Saccharum officinarum*.

**REFERENCES**


