APPLICATION OF MICROSATELLITE ANALYSIS TO THE SCREENING OF PUTATIVE PARENTS OF SUGARCANE CROSS AA40

S M HACK, B I HUCKETT and M K BUTTERFIELD

South African Sugar Association Experiment Station, Private Bag X02,
Mount Edgecombe, 4300, South Africa
E-mail: xtecbh@sugar.org.za

Abstract

In 1993 the genetic mapping of South African sugarcane cross, AA40, was initiated. However preliminary RFLP data suggested that the pedigree of the AA40 cross was incorrect. Mislabelling at planting or at seed collection was suspected as the cause of the invalid pedigree. Ten microsatellites (simple sequence repeats; SSRs) were used to analyse 13 potential parent cultivars and investigate the assertion of both mislabelling at planting (six potential parent pairs) and in a restricted manner that of mislabelling at seed collection (two potential parent pairs). The SSR primers chosen generated a total of 75 markers, of which 19 were monomorphic. The number of markers recorded per SSR primer pair across the 13 cultivars tested ranged between 2 and 11 with an average of 8. Although this work failed to identify the parents of the AA40 population, it did imply that the invalid pedigree of the AA40 population came about as a result of mislabelling at seed collection.

Keywords: sugarcane cross, parentage, microsatellites, SSRs

Introduction

A sugarcane genetic mapping project was initiated at SASEX in 1993, in order to identify quantitative trait loci (QTLs) related to important phenotypic characters. Six biparental crosses were planted in the field and the progeny of one cross, AA40 (N18 x CP57/614), that showed good segregation for a number of important traits, was selected for the mapping project. Phenotypic and DNA marker data have been collected in an ongoing effort to identify markers linked to traits that would be applicable, ultimately, in marker assisted selection. Although, at the outset of the mapping project, Random Amplified Polymorphic DNA (RAPD) analysis of the AA40 cross suggested that the parents were correct, subsequent Restriction Fragment Length Polymorphism (RFLP) data showed a relatively high proportion of non-parental bands in the progeny, indicating that the parents of the AA40 population were not N18 or CP57/614.

The incorrect pedigree of the AA40 progeny could have arisen as a result of mislabelling at planting or seed collection. In this investigation, microsatellite analysis was carried out on 13 potential parent cultivars and 10 AA40 progeny, and explored the assertion of both mislabelling at planting (six potential parent pairs) and in a restricted manner that of mislabelling at seed collection (two potential parent pairs).

Microsatellites or simple sequences repeats (SSRs) were chosen because they provide a higher incidence of detectable polymorphisms than other techniques such as RFLP and RAPD analysis, with greater reproducibility (Powell *et al.*, 1996; Jones *et al.*, 1997). In sugarcane, microsatellite primer pairs produce unique banding patterns for each cultivar. However, because of the high ploidy level in sugarcane, microsatellite data interpretation is complicated and often it is difficult to determine if one is detecting multiple alleles at one locus, or alleles at multiple loci. Here, the term marker will be used to refer to amplification products resolved on an electrophoresis gel.

Materials and Methods

Primers
The sugarcane SSR primers used were developed by the International Sugarcane Microsatellite Consortium (ISMC), of which SASEX was a participative member. This consortium produced more than 200 microsatellite primer pairs, as described by Cordeiro *et al*. (2000). Twenty-one primer pairs were preliminarily selected for this study based on the available polymorphic information content (PIC) data (Cordeiro *et al.*, 2000). The primers were diluted to 50µM with sterile distilled water and were stored at –20ºC.

DNA isolation
Fresh leafroll of 13 potential parent cultivars and 10 AA40 progeny, randomly selected from the population of 150 progeny, were collected and ground in liquid nitrogen and total DNA extracted following a modification of the procedure of Dellaporta *et al*. (1983).

Polymerase chain reaction
PCR amplification was carried out in a 25µl reaction volume, containing 50ng of DNA, 200 µM dNTPs, 0.2 µM of each primer, 2.5mM $MgCl_2$, 1.5 U Taq polymerase (Promega) and 1X PCR Buffer (10mM Tris-HCl pH 9, 50mM KCl and 0.1% Triton® X-100). The reactions were run on a GeneAmp 9700 thermocycler. Cycling conditions were: 94ºC for 5 min; followed by 35 cycles of 94ºC for 30 sec, appropriate annealing temperature (either 50ºC or 55ºC) for 30 sec, 73ºC for 30 sec and a final extension step of 73ºC for 3 min.

Amplification products were resolved using 8% (w/v) denaturing polyacrylamide/ 1X TBE gels and visualised by staining with SYBR® Gold (Molecular Probes).

Gel analysis and scoring
For each genotype, the bands on the gels were scored as either present (1) or absent (0), and the marker data of potential parental pairs was compared to that of ten randomly chosen progeny. Only parent pairs in which a particular marker was absent (0 x 0) could be regarded as discriminating. If present (1) in any of the progeny, these discriminating markers would constitute a mismatch between the parental pair and the progeny. In parentage analysis such a mismatch, referred to as a non-parental marker, would eliminate that parent pair from further consideration. This type of exclusion strategy is the only one possible in the case of a complex polyploid, such as sugarcane.

Results and Discussion

Twenty-one primer pairs were used to screen the 8 potential parent pairs (13 cultivars) and the 10 progeny. Ten SSR primer pairs of the twenty-one tested were found to generate polymorphic scorable banding patterns, and these primers were used for the parentage analysis. The ten SSR primer pairs generated a total of 75 markers, of which 19 were monomorphic. The number of markers recorded per microsatellite across the 13 cultivars tested ranged between 2 and 11 with an average of 8.

The total number of non-parental markers found across all microsatellites for each potential parent combination is shown in Table 1. For each parent combination, a significant number of non-parental markers were found in the progeny, indicating that none of these pairs are likely to be the true parents of AA40. It thus appears that the mislabelling of the cross did not occur at the time of planting, suggesting that mislabelling occurred when the cross was made. Future work will look at screening additional germplasm to identify the true parents.

Table 1. The number of discriminating* and non-parental markers found for each potential parent combination.

Parents	Cross Name	No. of discriminating markers	No. of non-parental markers in the progeny
Parental pairs included to investigate mislabelling at planting			
N18 x CP57/614	AA40	16	16
81W50 x N14	Z586	8	8
CP56/59 x Co1001	AA1312	14	13
NCo376 x 75F2297	AA1292	7	6
MZC74/275 x 77F790	Z927	15	14
79F2011 x N14	AA157	18	18
Parental pairs included to investigate mislabelling at seed collection			
N17 x CP57/614	N/A	14	13
MZC74/275 x 76F0879	N/A	16	9

* Discriminating markers are those from the total pool of markers absent in both parents. Incidence of such markers in the sample of 10 progeny (non-parental markers) indicates mismatch between parents and progeny.

This study has shown that microsatellites are an effective tool for studying problems of labelling and identification in sugarcane. Furthermore microsatellites could be applied as tools in other areas of sugarcane breeding programmes, such as (1) marker-assisted selection, (2) ensuring that field grown cane is true to type and (3) determining genetic diversity between commercial sugarcane cultivars. In order to achieve this, high throughput methods of DNA extraction and microsatellite processing need to be implemented in order to deal with increasing numbers of samples.

REFERENCES

Cordeiro, GM, Taylor, GO and Henry, RJ (2000). Characterisation of microsatellite markers from sugarcane (*Saccharum* sp.), a highly polyploid species. *Plant Science* 155: 161-168.

Dellaporta, SL, Wood, J and Hicks, JB (1983). A plant DNA mini-preparation: Version II. *Plant Molecular Biology Reporter* 1: 19-21.

Jones, CJ, Edwards, KJ, Castaglione, S, Winfield, MO, Sala, F, Vandewiel, C, Bredemeijer, G, Vosman, B, Matthes, M, Daly, A, Brettschneider, R, Bettini, P, Buiatter, M, Maestri, A, Malcevschi, N, Marmiroli, R, Volckaert, G, Rueda, J, Linacero, R, Vazquez, A and Karp, A (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* 3: 381-390.

Powell, W, Morgante, M, Andre, C, Hanafey, M, Vogel, J, Tingey, S and Rafalski, A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2: 225-238.