# PRELIMINARY GENETIC ANALYSIS OF THE SUGARCANE POPULATION AA40

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**Abstract**

The AA40 population has shown good segregation for various economically important characteristics. As such, SASEX is accumulating both molecular and phenotypic data on this cross in an attempt to generate a genomic map and discover DNA markers linked to measurable characteristics.

DNA samples from a selection of eighty AA40 progeny and the two putative parents were cut with two restriction enzymes, the samples purified and blotted on to nylon membranes. These were then probed with two sugarcane genomic DNA sequences which had been radioactively labeled.

From the large number of shared bands on these RFLP blots, it is evident that the AA40 progeny arose from a single crossing event. At the same time, there are sufficient polymorphisms between the individuals to make the cross suitable for molecular mapping.

RFLP results also showed that there were bands present in the progeny which were not present in either of the two parents, N18 or CP57/614. On closer examination it became clear that the male parent of the cross had been misidentified. Experiments are currently under way in an attempt to identify this parent.

## Introduction

The sugarcane population AA40, which arose from a cross between two commercial varieties, has shown good segregation for various economically important characteristics such as rust and smut resistance, to mention only two. SASEX is accumulating both molecular and phenotypic data on this cross with the purpose of generating a genomic map and identifying DNA markers linked to certain traits. To date, work in the Biotechnology department has focused on extraction of DNA from the putative parental varieties (N18 and CP57/614) and from all 150 progeny, and preliminary testing of this DNA to confirm that AA40 is a true cross. This assessment has involved Restriction Fragment Length Polymorphism (RFLP) analysis (Botstein *et al.*, 1980) in which polymorphic banding profiles are observed after DNA is cut with a restriction enzyme, the fragments separated on an agarose gel, transferred to a nylon membrane and then probed with homologous DNA sequences. This technique has been used extensively to map the genomes of various crops such as wheat (BenAmer *et al.*, 1997), barley (Bezant *et al.*, 1997) and maize (Ming *et al.*, 1997) as well as other sugarcane crosses (Grivet *et al.*, 1996).

## Materials and methods

### *DNA Extraction*

DNA was extracted from N18 and CP57/614 and the 150 progeny of AA40 using a methodology modified from Dellaporta *et al.* (1983). Leaf roll tissue (3-6 g) was frozen in liquid nitrogen and ground to a fine powder before being transferred to a sterile 50 ml tube containing 35 ml of extraction buffer (100 mM Tris-HCl (pH 8,0), 500 mM NaCl, 50 mM EDTA (pH 8,0) and 1% (v/v) β-mercaptoethanol). Tubes were shaken vigorously, after which 3,5 ml of 20 % (w/v) SDS was added. Samples were incubated at 70°C for 1 to 1,5 hours before 7 ml of 5 M potassium acetate was added and tubes incubated on ice for 20 min. After centrifugation (10 000 x g for 15-20 min), the supernatants were filtered through mutton cloth into fresh tubes (2 tubes per sample). Isopropanol (3/4 volume) was added to each of the samples and the tubes inverted several times before the DNA was spooled out using glass hooks. DNA was dissolved in 2 ml sterile water by incubation at 37°C overnight. Protein and other contaminants were removed by addition of an equal volume of chloroform : isoamyl alcohol (24:1), centrifugation at 4 000 x g for 10 min and removal of the upper aqueous phase.

DNA was then quantified and the integrity and purity of the extract determined as described previously (Harvey and Botha, 1996).

### *RFLP analysis*

Aliquots of DNA from N18, CP57/614 and a random selection of 80 progeny, were cut with two restriction enzymes, namely HindIII and BamHI. Reaction mixtures contained the following: 10 µg DNA, 1 x reaction buffer (Boehringer Mannheim), 4 mM spermidine and 30 U restriction enzyme, and were incubated at 37°C overnight. Contaminating RNA was removed by incubation of the samples with RNAse at 37°C for 30 min. Further purification to remove any remaining protein involved addition of an equal volume of phenol : chloroform : isoamyl alcohol (25:24:1), after which the tubes were shaken thoroughly and centrifuged at 100 x g for 10 min. The aqueous phase was then drawn off and the DNA precipitated by the addition of 150 mM NaCl and 2,5 x the volume of absolute ethanol. After solubilization in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8,0), restricted DNA samples were separated on 0,8% (w/v) agarose gels by

electrophoresis at 2 V/cm for 16-18 hours. Southern blotting was carried out according to Hoisington (1992).

The two sugarcane DNA probes used in these experiments, SSCIR 76 (obtained from a sugarcane leaf roll cDNA library) and SG 54 (isolated after enzyme restriction of sugarcane genomic DNA) were labelled with $^{32}$P-dCTP using the Mega-prime labelling kit (Amersham). All probe hybridisation and detection steps were carried out as described by Hoisington (1992).

## Results and Discussion

*Suitability of AA40 for mapping*

The results obtained in this preliminary study showed that the 80 AA40 progeny tested had similar, but not identical, RFLP banding profiles (Figure 1). This result is encouraging as it indicates that these individuals arose from a single crossing event, making the AA40 population suitable for genetic mapping. In addition, four of the fragments scored for presence/absence in the progeny appear to segregate in a 1:1 ratio indicating that this cross has sufficient polymorphism to generate markers using RFLP and other DNA analysis technologies.

*Identity of AA40 parents*

A result that was immediately apparent from the auto-radiographs, such as the example shown in Figure 1, was that there were bands present in the AA40 progeny (shown with arrows) which were not evident in either of the banding profiles obtained for the two putative parents. Due to the relatively higher percentage of band sharing with N18 and the improbability that the female parent would be mis-identified, this result implies that the male parent of AA40, thought to be CP57/614, had been misidentified at some stage prior to crossing, or during the crossing procedure itself. This was not unexpected, as there is ample opportunity for misidentification of parental material in the current breeding programme ([1]personal communication). This appears to be a common problem in other sugarcane breeding programmes. In fact, in up to 80% of sugarcane crosses carried out in Guadeloupe and Reunion (French West Indies), parents are incorrectly identified ([2]personal communication).

For any mapping exercise it is useful, but not essential, that the identity of both parents of the cross is known. Linkage may be determined by measuring the segregation ratio of a marker in the progeny, without analysing the parents. However, knowledge of parentage allows prior screening against only two parental samples to identify useful markers which are likely to segregate in the progeny. This could result in time and monetary saving in a long term mapping project. For this reason we are attempting to identify the unknown parent of AA40. At present DNA from all the other varieties, used as male parents in crosses conducted in the glasshouse at the same time as AA40, is being analysed using the RFLP technique described here.

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[2]Jean-Christophe Glaszmann, Agretrop, CIRAD, Montpellier, France

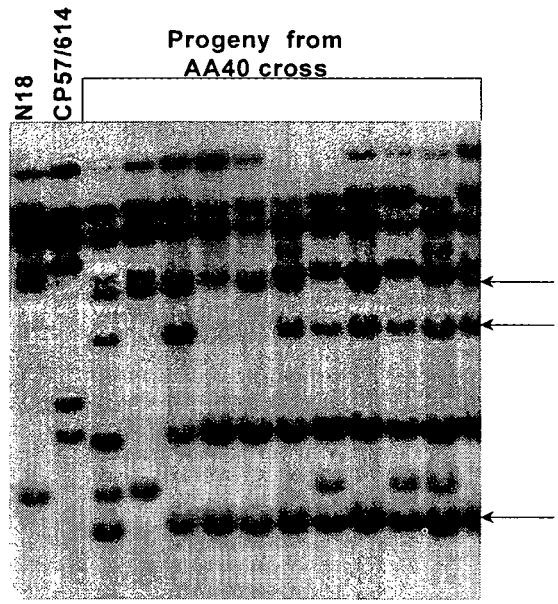


**Figure 1.** Representative portion of an autoradiograph showing the RFLP banding profile obtained with N18 and CP57/614 and a few progeny from AA40 (DNA restricted with HindIII and probed with SSCIR 76). Arrows show bands scored in the progeny, but absent in both N18 and CP57/614.

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