GENETIC MAPPING IN SUGARCANE: PROSPECTS AND PROGRESS IN THE SOUTH AFRICAN SUGAR INDUSTRY

JM BARNES AND AE BESTER

South African Sugar Association Experiment Station, Mount Edgecombe 4300

Abstract

Genetic mapping of a South African sugarcane cross, the AA40 population, was initiated with the ultimate aim of being able to identify genetic markers linked to important traits. This population is derived from a routine cross in the SASEX plant breeding programme, and was selected for mapping purposes due to the good segregation observed for various traits, for example resistance to diseases such as smut and rust. Initial molecular analysis of the progeny revealed that the identity of the male parent was not as originally supposed. Attempts were made to identify the true male parent, but these were unsuccessful. The inability to identify the male parent, however, is not crucial to the mapping project but merely limits the rate of accumulation of mapping data. Eighty AA40 progeny are being used for the generation of the map, whereby single-dose restriction fragment length markers are identified and used for linkage analysis. Although the linkage data generated to date are very much in the preliminary stages, the routine identification of single-dose markers will ultimately result in the definition of specific linkage groups. Phenotypic data of various traits can then be mapped, allowing the localisation of important genes in the sugarcane genome.

Introduction

Genome mapping is a powerful method used in the study of plant biology. A detailed genetic map contains a vast amount of information that plant breeders can use to identify, manipulate and complement traits to their maximum advantage, facilitating faster, more effective progress in crop improvement. Initially, genetic maps were developed using easily-scorable morphological markers, including natural or induced mutations, and isozyme markers. However, the development of molecular markers based on DNA polymorphisms revolutionised genome mapping and to date detailed genetic maps have been created for a great many plant species, for example rice (Kishimoto *et al*., 1993), soybean (Cregan *et al*., 1999), and sunflower (Gentzbittel *et al*., 1999).

Sugarcane (*Saccharum* species hybrids) is among the most genetically complex crop species. Commercial cultivars of sugarcane are highly polyploid, aneuploid, and of multispecific origin, and this complexity initially limited classical genetic studies in sugarcane (Hogarth, 1987) at a time when work with other more simple crops made significant gains. One exception to this was the work of Glaszmann *et al*. (1989), where isozyme variation was used to identify biochemical markers of potential utility in sugarcane genetics and breeding. This work illustrated how markers could be used as an efficient means of identifying linkage groups in a sugarcane genome. Large numbers of DNA markers, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA markers (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) can now be acquired with high efficiency on essentially any genome, and the application of these promoted the development of the theory and practice of genetic mapping of even the most complex of polyploids (Da Silva & Sobral, 1996).

The initial difficulty in mapping polyploids is largely due to the inability to identify the genotypes of markers based on their banding phenotypes, since there are a large number of possible genotypes for each marker in a segregating population (e.g. simplex Aaaa, duplex AAaa, triplex AAAa). To avoid this problem, Wu *et al*. (1992) proposed a method for mapping polyploids based on the segregation of single-dose restriction fragment length polymorphisms (SDRFs). A SDRF is produced at a single locus among all the homologous loci and is identified by its presence in one parent, absence in the other, and a 1:1 (presence : absence) segregation in the progeny.

Using the SDRF approach genetic maps were developed of Saccharum species involved in the ancestry of modern sugarcane (Da Silva *et al*., 1993, 1995; Al-Janabi *et al*., 1994). Genome mapping in modern sugarcane cultivars was initiated by Glaszmann's group at CIRAD (D'Hont *et al*., 1994), who were able to place 94 markers into 25 cosegregation groups. The same group later created a far more extensive map of another sugarcane cultivar, R570, and this consisted of 408 markers being placed in 96 cosegregation groups which were tentatively assembled into 10 basic linkage groups (Grivet *et al*., 1996).

The South African Mapping Population

The sugarcane population AA40 arose from one of the routine crosses of the breeding programme at SASEX. This population showed good segregation for various economically important characteristics (e.g. resistance to the diseases smut and rust), and was therefore selected as the population with which to generate a genomic map, the long-term goal of which would be the identification of genetic markers linked to certain traits. In view of this goal, SASEX is accumulating both phenotypic data and molecular data, the latter of which is described in this paper.

Eighty individuals of the AA40 population were randomly selected for mapping purposes and a preliminary RFLP analysis of these individuals was conducted to determine the suitability of the population for genomic mapping (Harvey & Huckett, 1998). These results confirmed that the individuals arose from a single crossing event, and they furthermore appeared to have sufficient genetic polymorphism to generate suitable markers. However, from this initial analysis it became evident that the male

parent of AA40, thought to be CP57/614, had likely been misidentified at some stage prior to crossing, or during the crossing procedure itself. Prior to the detection of markers for the construction of the genetic map, an attempt was made to resolve the true identity of the male parent.

Identity of the Male Parent

The RFLP hybridisation patterns of a subset of 10 AA40 progeny were compared to those of 14 putative males and the female N18. The putative males consisted of all those varieties used as males in crosses conducted in the breeding glasshouse at the time of the AA40 cross. The aim of this comparison was to determine whether a combination of one of the putative males with N18 could together account for the banding patterns observed in the progeny. Some of the putative males consistently showed patterns that were significantly different to those of the progeny. However, closer analysis of those that showed a higher similarity to the progeny could not result in the definitive identification of a single variety as the male parent of AA40.

For the construction of a genetic map it is useful, but not essential, that the identity of both parents of the cross is known. This allows preliminary screening of the parents to identify markers that are likely to segregate in the progeny, and could result in both time and monetary saving in a long-term mapping project. Since this is not possible with the AA40 project, the entire mapping population will have to be screened with each probe-enzyme combination used in order to detect the maximum number of useful SDRF markers.

Segregation Analysis and Preliminary Linkage Analysis

To date, RFLP analysis of the AA40 population has been done using 14 probes and two enzymes. Probes were obtained from CIRAD, Montpellier (SSCIR probes). These are sugarcane probes, prepared from a *S. spontaneum* genomic library. Selection of the fourteen probes was based on the map of sugarcane cultivar R570 (Grivet *et al*., 1996), with at least one probe for each of the 10 basic linkage groups. Of the 28 probe-enzyme combinations, 25 yielded distinguishable, scorable polymorphic fragments. A total of 80 polymorphisms were scored, of which 57 were found to be single-dose, i.e. conformed to a 1:1 segregation ratio by means of a χ^2 test ($p \geq 0.05$).

Table 1. Allocation of markers to 12 cosegregation groups using two-point linkage analysis with MAPMAKER 3.0. Markers are named according to the probe and enzyme used, with a number indicating, in decreasing size order, the position of the band in the RFLP pattern. For example, C77.H2 was generated using the probe SSCIR77 (C77) and the enzyme HindIII (H), and it was the second largest polymorphism (2).

Group	Markers
1	C60.H1; C60.D1; C119.D4
2	C119.H4; C119.D2
3	C77.H2; C83.H1
4	C77.H3; C77.D1; C83.H2; C83.D1
5	C77.H4; C83.H3
6	C77.D2; C83.D3
7	C77.D3; C83.D2
8	C101.H1; C101.D2
9	C101.H3; C101.D1
10	C217.H1; C217.D2
11	C217.D1; C217.D3
12	C256.H1; C256.D1

The construction of a genomic map is a statistical exercise performed using a programme such as MAPMAKER 3.0 (Lander *et al*., 1987), which assigns markers to linkage groups on the basis of their recombination values. Although 57 markers can by no means be used to generate a map, preliminary linkage analysis can be performed to determine which of the markers are linked together in groups, referred to at this stage as cosegregation groups. Grouping of these markers using two-point analysis with a LOD score of 3.0 resulted in 27 of the markers being assigned to 12 different cosegregation groups (Table 1), with the remaining 30 unassigned. Considering the polyploid nature of sugarcane, once a more saturated map has been created several groups of cosegregating markers may be found to correspond to the same basic linkage group.

From Table 1, it is encouraging to note that different markers generated by the same probe often group together. In the case of, for example, cosegregation groups 8 and 9, both groups contain markers generated by probe SSCIR101. Two possibilities exist for such an occurrence. The first is that these two groups may, in fact, be two separate cosegregation groups that correspond to one basic linkage group on the grounds that they share a common probe. The alternative is that they may both be part of a single cosegregation group, but because there are at this stage insufficient markers for a more complete linkage analysis, MAPMAKER was unable to find evidence for linkage of all four markers together at the specified linkage criteria.

Cosegregation group 1 contains markers produced by probes SSCIR60 and SSCIR119. This is not unexpected, since both these probes map to Linkage Group III of the R570 map of Grivet *et al*. (1996). Comparison of three different sugarcane maps (Da Silva *et al*., 1993; D'Hont et al., 1994; Grivet *et al*., 1996) has revealed a correspondence between some of the linkage groups defined in each of the studies.

Probes SSCIR77 and SSCIR83 map to Linkage Groups IV and X, respectively, of the R570 map (Grivet *et al*., 1996). The results reported here seem to contradict this (Table 1, groups 3-7), however it must be remembered that at this stage no conclusions can be drawn regarding the linkage groups of the AA40 map since to date only 14 probes have been used.

The next stage of the AA40 mapping project will be the identification of increased numbers of markers, using additional probes from various sources. These could be other sugarcane genomic probes including a new set of SSCIR probes to be provided by CIRAD; sugarcane expressed sequence tags (ESTs) such as those produced at SASEX (Carson *et al*., 1998); and probes from species such as maize which show synteny with sugarcane (Grivet *et al*., 1996). Increasing the number of markers will result in the more precise definition of linkage groups. Once a more informative map has been generated, phenotypic data of agronomically important traits can be mapped, allowing the location of important genes and quantitiative trait loci (QTLs) in the sugarcane genome. With this in mind, field trials

are continuing to accumulate data on traits such as resistance to diseases, sucrose accumulation and fibre content.

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