CTA024

FINAL REPORT

TITLE: MAP-BASED CHROMOSOME AND TRAIT TAGGING IN SUGARCANE USING CYTOLOGICAL AND RFLP MARKERS

PROJECT NUMBER: CTA024

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NON-TECHNICAL SUMMARY

Sugarcane is a complex aneuploid, polyploid, interspecific hybrid. At the time that this project began, molecular mapping in sugarcane was in its infancy and was only being attempted in interspecific crosses or within the selfed progeny of a variety. Two objectives of this project were, therefore, to demonstrate that markers could be used in variety by variety crosses in sugarcane and that molecular marker maps could be constructed in such crosses. Using a variety of marker types, these objectives have been met as described below.

The major aim of the project was to enable the Australian sugar industry to access overseas information on traits, markers and genome organisation in sugarcane and other grasses. This was to be achieved using two strategies. Firstly, other groups nationally and overseas have begun searching for markers linked to agronomic traits of interest and relevance to sugarcane. These markers, identified in sugarcane and related grasses such as sorghum and maize, might provide a more rapid means of identifying useful markers for the Australian sugarcane industry, as compared to searching for markers *de novo*, as had been done in all other sugarcane populations to date. The second strategy was to develop a framework map in an Australian sugarcane cross using these and other markers. This map could then be used as a means of both identifying new markers linked to traits of interest in sugarcane and of aligning Australian co-segregation groups with linkage groups, and the information contained within, identified in other maps of sugarcane and related grasses in other laboratories.

The population selected for analysis, Q117 x 74C42, was developed by Dr Nils Berding as part of BS138S. The population was grown with 4 replications at 2 sites over 2 years and evaluated for 8 traits - CCS, Brix, Pol, Fibre, Moisture, Stalk Number, Suckering and Stem Wax. The population was also screened for rust, however, insufficient infection was obtained and further analysis with this trait was not attempted.

Markers linked to sugar-related traits and to rust in sugarcane and sorghum were sought from Dr Andrew Paterson (16 probes), University of Georgia USA, and Dr Angelique D'Hont (2 probes), CIRAD France. Mapped "anchor" clones which hybridise to a diverse range of grasses including rice and sugarcane were requested from Dr Susan McCouch, Cornell University USA (72 probes) and well characterised and mapped sugarcane probes were requested from Dr Angelique D'Hont (50 probes), CIRAD France.

Of the markers linked to sugar-related traits and rust in sugarcane, only 7 of 16 probes from Dr Paterson and none from Dr D'Hont were useable in the BSES population Q117 x 74C42. Of the 7 probes from Dr Paterson, 4 were significantly linked to sugar-related traits in the BSES population (CCS, Brix and/or Pol) across all sites and years, or one or other site for both years. All 7 probes were significantly associated in one site/year, indicating that the same genomic regions are involved in sugar content in the interspecific and intervarietal crosses studied by Dr Paterson and by us, respectively.

Of the well-characterised and mapped markers from sugarcane and other species, approximately 30 were found to be useable. These probes generated approximately 60 markers which could be mapped and provide points of reference between this map and information developed in this project and maps and information developed in other sugarcane and grass mapping laboratories. In addition, the RAF technique, developed by Dr Bernie Carroll, UQ, as part of BSCS1, was used as a means of rapidly producing markers. Ten primers were used to generate almost 200 markers.

Linkage analysis was performed using both the RAF and RFLP markers. Strong linkage was found between markers and all traits evaluated (CCS, Brix, Pol, moisture, stalk number, stalk wax, suckering), using single factor analysis. A total of 12 markers were found that were significantly associated with one or more traits across both years and both sites. These “robust” markers explained varying percentages of the variation in a trait in the population. In general, only small amounts of variation in the sugar-related traits were explained by each marker, suggesting many QTLs of individually small effect. The maximum level of variation explained by an individual marker was 20% for suckering. This marker was linked to a 1.5 difference in sucker number and a corresponding loss of 0.2 of a CCS unit and 0.42 of Brix and was one of the probes identified by Dr Paterson as linked to sugar-related traits in his crosses. Other important markers were found for fibre (16%), stalk number (14, 16 and 17%) and suckering (17%). Markers were frequently linked to more than one trait, such as Brix, Pol and CCS, as expected, and Brix, Pol, CCS and suckering, indicating the importance of reviewing the entire data set in addition to trait by trait.

Other markers were identified that were significantly associated with traits at one site over both years. However, the 2 years data are not independent as the phenotypic data was obtained in Year 2 on the ratoon crop. Given the site differences, it is not unexpected that “site-specific” markers should be identified. These markers will be evaluated further to verify their association.

We have identified several markers that are significantly associated with suckering. We have been able to locate these onto the sorghum map of Dr Andrew Paterson and to our interest, have found that they co-locate to sorghum genomic regions associated with rhizomatousness and tillering of sorghum. This result strongly supports the use of sorghum as a reference map, to simplify mapping in sugarcane.

To date, we have mapped approximately 160 markers. These markers, comprising 59 markers from 26 RFLP probes and 199 markers from 10 RAF primers, have formed a sugarcane map with 30 co-segregation groups. By using the RFLP probes as anchors, we have been able to partially align our co-segregation groups with the maps from TAMU and CIRAD. We have co-segregation groups that align with the 10 basic linkage groups of the TAMU and CIRAD maps. Many of the co-segregation groups in our map are without RFLP markers and hence cannot be aligned. More markers are required to link these groups to co-segregation groups with RFLP markers and will be done as part of CTA049, using microsatellites.

PROJECT BACKGROUND

Sugarcane is a complex aneuploid, polyploid, interspecific hybrid. At the time that this project began, molecular mapping in sugarcane was in its infancy and was only being attempted in interspecific crosses or within the selfed progeny of a variety or ancestral species. In addition, the only traits being targetted were sugar-related traits or simple diseases such as rust and eyespot. All of these traits are relatively easy to measure in the field and have high heritability and therefore re not prime targets for marker-assisted selection. Additional objectives of this project were, therefore, to demonstrate that markers could be used in variety by variety crosses in sugarcane and that molecular marker maps could be constructed in such crosses, and that more complex and useful traits could be tagged in sugarcane. Using a variety of marker types, these objectives have been met as described below.

The major aim of the project was to enable the Australian sugar industry to access overseas information on traits, markers and genome organisation in sugarcane and other grasses. This was to be achieved using two strategies. Firstly, other groups nationally and overseas have begun searching for markers linked to agronomic traits of interest and relevance to sugarcane. These markers, identified in sugarcane and related grasses, might provide a more rapid means of identifying useful markers for the Australian sugarcane industry, as compared to searching for markers *de novo*, as had been done in all other sugarcane populations to date. The second strategy was to develop a framework map in an Australian sugarcane cross using these and other markers as a means of both identifying new markers linked to traits of interest and of aligning Australian co-segregation groups with linkage groups, and the information contained within, identified in other maps of sugarcane and related grasses.

Molecular markers have the potential to greatly assist plant breeders through marker-assisted selection, fingerprinting of varieties, charaterisation of germplasm etc. Detailed genetic maps are currently available for most of the major agricultural species. It has recently been found that there is considerable conservation of gene and marker order amongst the grass species. Work is currently underway in several laboratories to align the many genomes of the Pooideae grasses using “anchor” probes which have already been mapped in several species and hybridise across a wide range of grass species. Comparative mapping is being used to “fill-in” gaps on specific linkage maps. Of particular interest, however, is recent research in maize and rice demonstrating the use of comparative mapping to predict the location of a gene (pistillate) in rice, based on the known location of similar gene in maize (tassel seed). This approach could be extended in sugarcane to look for genes controlling agronomic traits, such as drought resistance, using markers which are linked to known components of drought resistance in other species, such as staygreen in sorghum. A set of “anchor” probes has been produced by Cornell University to enable all workers to align their linkage groups/maps with each other by identifying linkage groups with probes in common.

OBJECTIVES

1. To test markers identified by other laboratories in the USA and France as linked to agronomic traits of interest in sugarcane (including rust, Brix, propensity to flower, and height) in Australian populations, to see if these markers are linked to the genes for these traits which are segregating in these populations.

Achieved: Markers were requested and received from several labs that had been identified as linked to traits which were segregating in the BSES population. These traits included sugar-related traits, suckering, tillering, rust and height. However, phenotypic data was only obtained for the first three of these traits. Although attempted for two years, it was not possible to obtain a good rust infection and height was too variable within clones to enable accurate measurement of this trait. The RFLP markers obtained were screened for SDRF polymorphisms and polymorphic markers screened over the population progeny. Significant associations were found for several of the sugar-related RFLP markers from the laboratory of Dr Andrew Paterson, indicating that the same genomic regions are involved in his interspecific and our intervarietal cross.

2. To enable Australian sugarcane researchers to access trait, marker and genome organisation information in other grasses by producing a skeleton linkage map in an Australian sugarcane cross using "anchor" RFLP markers from other crop species to correlate specific sugarcane linkage groups with linkage groups in the grasses and other sugarcane maps.

Achieved: We have produced a skeleton linkage map with approximately 30 co-segregation groups which includes 59 RFLP markers and 199 RAF markers. More than twenty-five RFLP probes were used to obtain the 59 RFLP markers and most of these probes have been mapped in other species, including sugarcane. We have been able to align many of our co-segregation groups with other sugarcane and sorghum maps, using these markers. Evidence of the usefulness of this approach is demonstrated by the observation that many of the RFLP markers which we have identified as linked to suckering in sugarcane co-locate to known tillering/suckering regions in sorghum.

3. To position the markers identified in BS138S which are linked to agronomical traits onto the linkage groups.

Not achieved: The original aim of BS138S was to use a Bulked Segregant Approach to identify markers linked to rust resistance in the 2 BSES populations. These markers would then be mapped in this project. As mentioned before, despite several attempts, it has not been possible to obtain sufficient rust infection on these populations and so markers have not been identified. BS138S has been extended to enable other populations to be evaluated for rust prior to BSA being performed. Any resulting markers will be incorporated into our later maps.

4. To provide breeders with other tightly linked markers (RFLPs) for traits segregating in the populations characterised in BSES138S to increase the possibility of obtaining closely linked markers which are polymorphic in other crosses in addition to the crosses in which they were identified.

Achieved: Strongly significant associations have been found between markers, both RFLPs and RAFs, and all 8 traits measured. These markers explain varying amounts of the variation in the traits. In general, only small amounts of variation (1-10%) in the sugar-related traits were explained by each marker, suggesting many QTLs of individually small effect. The maximum level of variation explained by an individual marker was 20% for suckering. Other important markers were found for fibre (16%), stalk number (14, 16 and 17%) and suckering (17%).

5. To isolate repetitive specific sequences for *S. officinarum* and *S. spontaneum* and use to "tag" and identify specific sugarcane chromosomes.

Partially achieved: After initial isolation of potential candidate sequences and their preliminary characterisation, this milestone, and Objective 6, were discontinued, for several reasons. Firstly, mapping was expanded to include a greater number of progeny. Secondly, it proved more difficult than initially expected to identify RFLP probes with SDRF polymorphisms. More time was expended on this activity than initially expected. Thirdly, after attending the ICSB meeting in San Diego in 1997, it was realised that a PhD student at CIRAD was focussing on this area of research, and fourthly, with the advent of BAC libraries and *in situ* hybridisation with BACs, the use of repetitive sequences to identify specific chromosomes was passé.

6. To locate these sequences on the genetic linkage map and associate specific chromosomes with specific linkage groups.

Not achieved: See above.

METHODOLOGY

- **Phenotypic Trait Evaluation**

Five sugarcane populations were developed by Dr Nils Berding, BSES, as part of BS138S and progeny from each were evaluated in the field. After visual appraisal of the 5 populations, two were selected for further analysis, Q117 x 74C42 (312 progeny) and Q96 x Q173 (170 progeny) because both populations had large numbers of progeny and were vigorous in the field. Both populations were grown with 4 replications at 2 sites over 2 years and evaluated for 8 traits - CCS, Brix, Pol, Fibre, Moisture, Stalk Number, Suckering and Stem Wax. The phenotypic data obtained and the methodology used to measure the phenotypes are described in the BSS138 Final Report.

Both populations were also screened for rust, however, insufficient infection was obtained and analysis with this trait was not attempted.

In this project (CTA024), mapping and linkage analysis were carried out on the Q117 x 74C42 population only.

- **DNA Isolation**

DNA was isolated using previously described methods. For RFLP analysis, DNA was isolated as described in the Laboratory Protocols Manual developed by CIMMYT (Hoisington, DA. Laboratory Protocols. CIMMYT Applied Molecular Genetics Laboratory. Mexico, D.F.).

- **RFLP and RAF Protocol**

All techniques relevant to RFLP analysis; including restriction enzyme digestion, probe preparation, hybridisation and autoradiography were carried out as described previously in BSCS1 and the Laboratory Protocols Manual developed by CIMMYT (Hoisington, DA. Laboratory Protocols. CIMMYT Applied Molecular Genetics Laboratory. Mexico, D.F.).

All techniques relevant to RAF analysis; including PCR, gel separation and autoradiography were carried out as developed by Dr Bernie Carroll, UQ and described previously in BSCS1.

- **Linkage Analysis**

One hundred and eight progeny were screened for the presence or absence of each RFLP or RAF marker band. Each progeny was checked to ensure that all bands scored originated from one of the 2 parents, Q117 or 74C42, M0 used to designate bands originating from 74C42, M1 bands from Q117, and M2 to designate segregating bands present in both parents. Clone 43 was identified as having markers that were not present in either parent. This individual was excluded from the rest of the analysis. All of the remaining 107 clones had some male specific bands indicating that they were not the result of self-pollination of the female parent.

A total of 258 markers were scored, including 59 RFLP and 199 RAF markers. Of these, 163 had segregation ratios that did not deviate significantly from 1:1, suggesting that they were single dose markers. These markers were used for linkage analysis.

The software package JoinMap (Stam and Van Ooijen 1995) was used for linkage analysis, incorporating the following parameters. The population was scored as population type CP (cross pollination). A lod threshold of 4.0 was used for grouping the markers. The linkage analysis resulted in 93 of the 163 markers showing linkage with at least one other marker in 30 linkage groups. Due to the small number of linkage groups and the large number of markers which showed segregation which differed from 1:1 single factor QTL analysis was applied to the data set using the Kruskal-Wallis test option.

RESULTS

1. To test markers identified by other laboratories in the USA and France as linked to agronomic traits of interest in sugarcane (including rust, Brix, Pol) in Australian populations, to see if these markers are linked to the genes for these traits which are segregating in these populations

Where possible, we have attempted to compare QTLs from the sugarcane maps of TAMU and CIRAD with our QTLs, as these QTLs are the most relevant. Unfortunately, we have not been able to compare the QTLs for Brix from CIRAD, as the CIRAD probes linked to these QTLs are AFLP markers and hence not cross transferable.

We have had more success with the QTL probes from TAMU. Of the set of 29 probes that Dr Andrew Paterson identified as linked to sugar traits in his *S. officinarum x S. spontaneum* cross, 7 were polymorphic in our cross. Four of the seven are significantly linked to sugar related traits (CCS, Brix and/or Pol) in our cross in both sites and years (all) or both years at a single site (M-Meringa; B-Bellinden Ker), indicating the same regions are involved in the traits in both the wild relatives and elite varieties (Table 1). The remaining 3 markers, in addition to the other 4, were significantly associated in a single site/year (SS). One of these probes (PSB103) is also significantly negatively associated with suckering (see below).

For both the CIRAD and the 22 other TAMU QTLs, further work is planned as part of CTA049 to identify probes located near to these markers which may be polymorphic in our cross. These markers will then allow us to test if more sugar-related QTLs are common to these three mapping efforts.

Table 1. Results from screening with probes from Dr A Paterson

	CCS				Brix				Pol			
RFLP Probe	All	M	B	SS	All	M	B	SS	All	M	B	SS
CDSC005	-	-	-	Y	*	*	-	Y	-	-	-	Y
CDSC042	-	-	-	Y	-	-	-	Y	-	-	-	Y
CDSC052	-	-	-	Y	-	*	-	Y	-	-	-	Y
CDSR015	-	-	-	Y	-	-	-	Y	-	-	-	Y
PSB103	-	*	-	Y	**	****	-	Y	*	**	-	Y
PSB82	-	-	-	Y	-	-	-	Y	-	-	-	Y
CDSB32	-	-	-	Y	-	**	-	Y	-	-	-	Y

All – significant association across both sites and years
M – significant association at Meringa in both years
B – significant association at Bellinden Ker in both years
SS – significant association at one site in one year
* (P<0.1); ** (P<0.05); ***(P<0.01); ****(P<0.005)

2. To enable Australian sugarcane researchers to access trait, marker and genome organisation information in other grasses by producing a skeleton linkage map in an Australian sugarcane cross using "anchor" RFLP markers from other crop species to correlate specific sugarcane linkage groups with linkage groups in the grasses and other sugarcane maps.

Five sugarcane populations were developed by Dr Nils Berding, BSES, as part of BS138S and progeny from each were evaluated in the field. After visual appraisal of the 5 populations, two were selected for further analysis, Q117 x 74C42 (312 progeny) and Q96 x Q173 (170 progeny) because both populations had large numbers of progeny and were vigorous in the field. Both populations were grown with 4 replications at 2 sites over 2 years and evaluated for 8 traits - CCS, Brix, Pol, Fibre, Moisture, Stalk Number, Suckering and Stem Wax. Each trait was evaluated using protocols developed by BSES. Only the Q117 x 74C42 population was studied in CTA024. The individual clonal phenotypes for all 8 traits, at both sites and in both years, are described in the Final Report for BSS138S.

A skeleton linkage map has been constructed in this population using 108 progeny. This number of progeny was selected as being sufficient to detect a range of variously sized QTLs. Significantly larger numbers of progeny need to be evaluated to detect QTLs of small effect.

To date, we have screened almost 260 markers in 108 progeny from the cross between Q117 x 74C42 (Table 2). These markers, comprising 59 markers from 26 RFLP probes and 199 markers from 10 RAF primers, have formed a sugarcane map with 30 co-segregation groups using 93 makers. A further 70 markers remain unlinked, while the remaining 95 gave non-1:1 segregation patterns. We have used RAFs rather than AFLPs as we have found RAFs to

be easier to use and they have no IP associated with them. Both techniques appear to give similar levels of polymorphism.

Table 2. Number, type and source of markers screened and mapped

Category	RFLP	RAF	Total
No of polymorphic probes/primers	26	10	-
No of markers scored	59	199	258
No of dominant markers from Q117	31	72	103
No of dominant markers from 74C42	26	62	88
No of monomorphic markers which segregated in the progeny	NS	65	65
Number of single dose markers 1:1	59	104	163
Number of linked markers	32	61	93

Progeny clone 43 was identified as having markers that were not present in either parent. This individual was excluded from the rest of the analysis. All of the remaining 107 clones possessed only bands present in Q117 or 74C42 and all possessed some male specific bands indicating that they were true hybrids between the two parents.

Of the 258 markers generated, 163 markers had segregation ratios that did not deviate significantly from 1:1. These markers were used for linkage analysis, using the software package JoinMap (Stam and Van Ooijen 1995). The population was scored as population type CP (cross pollination). A lod threshold of 4.0 was used for grouping the markers. The linkage analysis resulted in 93 of the 163 markers showing linkage with at least one other marker in 30 linkage groups (Table 3). The remainder of the markers were unlinked.

Table 3. Number of markers per linkage group (LG)

LG size (number of markers)	Number of linkage groups
2	17
3	2
4	6
5	3
6	1
8	1

Many RFLP markers gave more than one polymorphic band. These markers were usually linked to other, different markers, in different co-segregation groups. In this way, the 30 so-segregation groups could be reduced to 24 homology groups, with the largest homology group containing 12 markers (Homology Group 7, Table 4).

Table 4. Relationship between Linkage Groups and Homology Groups identified in this study and the Sorghum (SO) LGs from Paterson and CIRAD and the Sugarcane (SC) LGs from CIRAD.

CSIRO LG	Homology Group (HG)	No. Markers	Marker Common to HG	Paterson SO LG	CIRAD SO LG	CIRAD SC LG
1	1	6	SSCIR51	B	F	X
2	2	4				
3	3	4				
4	4	2				
5	5	2		A	G	II,III
6	6					
7	7	8	UMC6	D	B	VIII
8	8	5		F	D	VII
9	9	2				
10	10	5				
11	11	2		B	F	X
12	12	2	SSCIR103, 110	A	G	II,III
13	13	2				
14	14	5				
15	15	4		C	C	V,VI
16	16	3				
17	17	3				
18	7	2	UMC6	D	B	VIII
19	18	2				
20	20	4	PSB82, CDSB32	G F	E D	IV, X
21	20	2	CDSB32	F	D	X
22	19	2				
23	21	2				
24	7	2	UMC6	D	B	VIII
25	22	4				
26	20	2	PSB82	G	E	IV
27	23	2				
28	12	4	SSCIR103,110	A	G	II,III
29	24	2				
30	1	2	SSCIR51	B	F	X

By using the RFLP probes as anchors, we have been able to partially align our co-segregation groups with the sugarcane maps from TAMU and CIRAD (Table 4). We have screened our population with probes that span the 10 basic linkage groups in sorghum and sugarcane. We have co-segregation groups that align with the 6 of the 10 basic linkage groups of the TAMU and CIRAD maps (Table 4). Many of the co-segregation groups, however, are without RFLP markers and hence cannot be aligned and many of our

polymorphic markers, including mapped RFLP markers, are unlinked as yet. More markers are required to link these unlinked markers and unassigned groups to co-segregation groups with RFLP markers. This will be done as part of CTA049, using microsatellites, RAFs and other RFLP probes.

It has not been possible to align our map with the *S. officinarum x E. arundinaceus* map from Copersucar. Mapping this population has become a very low priority at Copersucar, given their staffing and financial difficulties. Only 10 markers have been identified in this population at Copersucar to date. We have more than 30 RFLP markers scored on this cross already, and approximately 50 RAF markers. The population has not been analysed further.

3. To position the markers identified in BS138S which are linked to agronomical traits onto the linkage groups.

As described above, this has not been achieved as the lack of adequate rust infection in these populations has prevented the original aims of BS138S to be achieved. Thus, as no marker(s) has been identified as part of BS138S, it is not possible to map them here. BSS138 has been extended to enable other populations to be evaluated for rust prior to BSA being performed. Any resulting markers will be incorporated into later maps, as part of CTA049.

4. To provide breeders with other tightly linked markers (RFLPs) for traits segregating in the populations characterised in BSES138S to increase the possibility of obtaining closely linked markers which are polymorphic in other crosses in addition to the crosses in which they were identified.

Phenotypic data

Phenotypic data was collected for eight traits at two sites, Meringa and Bellenden Ker, for a plant crop (1997) and ratoon crop (1998), with 4 replications for the 312 progeny of the Q117 x 74C42 cross.

Data was collected for the following traits at each harvest: Brix (g/kg), CCS (g/kg), Fibre (g/kg), Moisture(g/kg), Pol (^{o}Z)., Stalks per stool, Suckers per stool, Wax rating (subjective 1-5 rating).

The phenotypic score for each clone at each site and in each year is given in the BSS138 Final Report. The population mean and clonal range, from the BSS138S Final Report, for all 8 traits is given in Table 5.

Table 5 – Range of clonal means, Q1 population mean for all 8 traits measured in the Q1 population from combined analyses of variance, over locations and crops (BSS138 Final Report).

Trait	Clonal Mean Range	Population Mean
Brix	186.7-228.24	211.0
CCS	118.26 – 169.79	149.6
Pol	68.3 – 89.69	80.4
Moisture	670.58 - 732.28	698.3
Fibre	98.43 – 156.6	121.8
Stalk Number	3.86 – 16.31	9.1
Sucker Number	1.5 – 8.83	5.1
Stem Wax	1.56 – 4.19	3.2

Correlation Analysis

Correlation analysis was performed on the combined site x year data for each trait to examine the relationships and possible interactions between the traits evaluated. The results are given in Table 6.

As expected, the correlation coefficient between Brix, Pol and CCS was high and ranged from 0.87 to 0.97. The correlation was highest between Brix and Pol and lower for Brix or Pol and CCS.

Moisture was strongly negatively associated with Brix (-0.70) and Pol (-0.61) and less strongly negatively associated with CCS (-0.35). Moisture and fibre were also strongly negatively associated (-0.78).

Stalk number and suckering were strongly positively correlated (0.73) but showed little correlation with the other traits evaluated. Stem wax was weakly correlated with fibre (0.14) and stalk number (0.12).

Table 6. Correlation between traits using combined site x crop/year data.

	Brix	**CCS**	**Fibre**	**Moist.**	**Pol**	**Stalks**	**Suck**
Brix	1.00	0.87	0.11	-0.70	0.97	-0.05	-0.04
CCS	0.87	1.00	-0.28	-0.35	0.96	-0.08	-0.07
Fibre	0.11	-0.28	1.00	-0.78	-0.01	0.09	0.03
Moist.	-0.07	-0.35	-0.78	1.00	-0.61	-0.04	-0.01
Pol	0.97	0.96	-0.01	-0.61	1.00	-0.06	-0.06
Stalks	-0.05	-0.08	0.09	-0.04	-0.06	1.00	0.73
Wax	0.04	0.07	0.14	-0.11	0.07	0.12	0.07

QTL Analysis

Single Factor Analysis

Preliminary QTL analyses have been undertaken with the 258 markers and site x year combinations for all 8 traits, using the software package MapQTL (Stam and Van Ooijen 1995). Due to the small number of linkage groups and the large number of markers that showed segregation that differed from 1:1, single factor QTL analysis was applied to the entire data set using the Kruskal-Wallis test option.

Linkage was found between markers, both RFLP and RAF, and all traits measured (CCS, Brix, Pol, fibre, moisture, stalk number, stalk wax, suckering) (Appendix 1). Level of significance is indicated by the number of stars (1 star = P<0.1; 2 stars = P<0.05; 3 stars = P<0.01; 4 stars = P<0.005; 5 stars = P<0.001). The type of marker, parental source, linkage group location in Dr A. Paterson's sorghum map or Dr Grivet's sugarcane map and in the CSIRO sugarcane map is also given in Appendix 1.

Many markers are significantly associated with a trait across both sites and years (plant and ratoon crop), while other markers were associated with a trait at one site for both years, or at one site in a single year. The lack of correspondence of some QTLs across all sites is not unexpected and has been observed by various authors. This type of result may be caused by

- GxE interaction
- Variation in heritability across sites
- Sample variation due to small population size
- They may be site-specific and relate to site differences.

The small population size is likely to be the major cause of this variation in this situation, given the small size of many of the QTLs. Again, this will be explored further in CTA049, where the full 300 individuals of this cross will be analysed with these markers.

Significant Marker Associations Across Both Sites and Crops

Markers which were highly significantly associated with any of the traits across both sites and both crops/years were identified and are listed in Table 7.

In general, only small amounts of variation in the sugar-related traits were explained by each marker, suggesting QTLs of individually small effect. This is consistent with other studies (A.H.Paterson – ICSB Final Report, L. Grivet – pers. comm.). For Brix, individual markers explained up to 10% of the variation, and up to 10 and 7% of the variation in Pol and CCS. Markers strongly associated with one trait were usually strongly correlated with the other 2 traits (Table 7).

The maximum level of variation explained by an individual marker was 20% for suckering. Other major important markers were found for fibre (16%), stalk number (14, 16 and 17%) and suckering (17 and 20%).

For suckering, 2 major markers were identified, explaining 17 and 20% of the variation in this population, and both markers were also significantly associated with stalk number. An additional marker explaining 8% of the variation in the suckering, was also identified and also associated with stalk number. Stalk number had an additional major marker that explained a further 16% of the variation in this trait.

For moisture, 2 major markers each explaining approximately 11% of the variation and a minor marker explaining 7% of the variation was found and all 3 markers were significantly associated with CCS, Brix and Pol. One of the markers was also strongly associated with stalk number and suckering, while another was also associated with fibre. An additional minor marker for moisture was also a major marker for stalk number.

For stem wax, 2 markers each explained about 9% of the variation in this trait

Table 7. Markers significantly associated with "all traits" ie combined value across sites and crops/years.

R square	**Trait**							
Marker	**Brix**	**Pol**	**CCS**	**Moist.**	**Fibre**	**Stalks**	**Sucker.**	**Wax**
MOAB07x2	6%	5%	2%	11%	7%	1%	1%	0%
MOK09X47	1%	1%	2%	0%	1%	6%	8%	0%
MOK10X2	10%	10%	7%	7%	1%	3%	3%	0%
MOK17X13	0%	0%	1%	9%	16%	0%	0%	0%
MOSSCIR172E2	6%	6%	7%	0%	2%	1%	2%	2%
M1K09X9	0%	0%	1%	0%	1%	17%	17%	1%
M1K13X7	2%	2%	1%	3%	2%	16%	3%	3%
M1K17X28	8%	7%	5%	6%	1%	2%	1%	0%
M1PSB103E1	9%	5%	2%	11%	5%	14%	20%	4%
M1SUSI2E3	1%	1%	1%	1%	1%	1%	0%	9%
M2K09X115	0%	1%	1%	0%	0%	7%	4%	0%
M2M05X102	2%	2%	1%	5%	3%	1%	0%	9%

Phenotypic value of individual markers

Table 7 lists the markers which were found to be significantly associated with a trait over both sites and both crops/years. Other markers were also found which were significantly associated with a trait at one site over both crops/years.

For each marker, the progeny of the Q1 population were divided into 2 groups based on the presence or absence of the marker. The phenotypic mean was then calculated for each group (Progeny A and Progeny B means), to determine the approximate phenotypic value of each marker.

Markers designated M0 derive from 74C42, while markers designated M1 derive from Q117. Hence, for M0 and M1 markers, the progeny A mean is derived from progeny with marker genotypes similar to 74C42, while the

progeny B mean is derived from progeny with marker genotypes similar to Q117. The phenotypic contribution of a marker was determined to be positive or negative depending on whether the progeny mean value of progeny with the marker was greater than (positive) or less than (negative) than the progeny mean of progeny without the marker. Thus for an M0 marker, if the progeny A mean is greater than the progeny B mean, the marker is associated with a positive effect on the trait. If the marker is from Q117, a M1 marker, it is associated with a positive effect on the trait if the progeny B mean is greater than the progeny A mean.

For M2 markers, which derive from markers present in both parents, the progeny A mean is derived from progeny with the band. Thus, if the progeny A mean is greater than the progeny B mean, the M2 marker is associated with a positive effect on the trait.

A. Brix

Brix measurements are in g per kg. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138), ranged from 186.7-228.24, with a mean value of 211.0 (Table 5).

From Table 7, 9 markers are significantly associated with Brix over locations and crops/years and these are listed in Table 8A. Three markers were identified that explained 8-10% of the variation in this trait in the Q1 population. This level of variation correlates with approximately 4 g/kg, that is, on average, progeny with the marker differed from progeny without the marker by 4g/kg. One marker was positively correlated with Brix, while two markers were negatively correlated.

Three additional markers were strongly correlated with Brix at Bellenden Ker only in both years (Table 8B). These markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Four of the markers associated with Brix have been mapped to sugarcane and sorghum linkage groups. Markers M0K10-2,and M0SSCIR51-E-2 are linked on Sugarcane Linkage Group 1 (Appendix 1, Table 4) while M1SSCIR51-E-1 is on linkage group 30. SSCIR51 maps to sorghum linkage group B. Thus, these 3 markers may be detecting the same genomic region or this linkage group may contain more than one QTL for this trait. Marker M1K13-7 maps to sugarcane linkage group 14. This linkage group does not have a sorghum equivalent at present.

Table 8 – Phenotypic value of markers associated with Brix.

A: Markers associated across both sites and crops

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	6%	210.0	213.0	-3.0
MOK09X47	1%	211.6	210.2	1.4
MOK10X2	10%	208.8	213.0	-4.2
MOSSCIR172E2	6%	209.2	212.5	-3.3
M1K13X7	2%	211.1	209.1	-2.0
M1K17X28	8%	212.5	208.4	-4.1
M1PSB103E1	9%	207.8	212.0	4.2
M1SUSI2E3	1%	210.0	211.1	1.1
M2MO5-102	2%	210.1	212.4	-2.3

B: Markers associated with single sites (B=Bellinden Ker)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
M1SSCIR51-E-1	*** B	202.0	205.1	3.1
M1K17-25	*** B	201.1	205.7	4.6
M0SSCIR51-E-2	*** B	201.3	207.4	6.1

B. CCS

CCS measurements are in g per KG. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 118.26-169.79, with a mean value of 149.6 (Table 5).

From Table 7, 10 markers were found to be significantly associated with CCS over locations and crops/years and these are listed in Table 9A. Three markers explained 5-7% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 3.4-4.1 g/kg difference (or 0.3-0.4 CCS unit). All 3 markers were associated with a loss in CCS.

Five additional markers were strongly correlated with CCS at either Meringa or Bellenden Ker in both years (Table 9B). Again, these markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Six markers associated with CCS have been mapped to sugarcane and sorghum linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 (Appendix 1, Table 4) while M1SSCIR51-E-1 is on Linkage Group 30. However, SSCIR51-E-2 is also on linkage group 1, suggesting that Linkage Groups 1 and 30 belong to the same homology group. Thus, these 2 markers may be detecting the same genomic region or this linkage group may contain more than one QTL for this trait. SSCIR51 maps to sorghum linkage group B. Marker M1UMC6-D-3 is on linkage group 7. This sugarcane linkage group has homology to a different sorghum chromosome, suggesting that this marker is detecting a different genomic region (Table 4).

Table 9. - Phenotypic value of markers associated with CCS.

A: Markers associated across both sites and crops/years

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	2%	148.7	152.2	-3.5
MOK09X47	2%	150.4	148.1	2.3
MOK10X2	7%	147.3	151.4	-4.1
MOK17-13	1%	150.0	148.5	1.5
MOSSCIR172E2	7%	147.1	151.1	-4.0
M1K09-9	1%	148.3	149.5	1.2
M1K13X7	1%	150.0	148.5	-1.5
M1K17X28	5%	150.7	147.3	-3.4
M1PSB103E1	2%	147.5	149.6	2.1
M1SUSI2E3	1%	148.5	149.7	1.2
M2K09-115	1%	149.5	146.0	3.5
M2MO5-102	1%	148.8	150.4	-1.6

B: Markers associated with single sites (B=Bellinden Ker; M=Meringa)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
MOK05-2	*** B	143.0	147.5	-4.5
M1K17-25	*** B	143.3	147.9	4.6
M1SSCIR51-E-1	*** B	142.9	147.3	4.4
M1UMC6-D-3	** M	154.8	148.6	-6.2
M1SSCIR69-D-1	*** M	155.7	149.4	-6.3

C. POL

Pol measurements are in degrees. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 68.3-89.69, with a mean value of 80.4 (Table 5).

From Table 7, 10 markers were found to be significantly associated with CCS over locations and crops/years and these are listed in Table 10A. Five markers explained 5-10% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 1.6-2.5 degree difference. Some markers were positively correlated with Pol, while other markers were negatively correlated.

Three additional markers were strongly correlated with Pol at either Meringa or Bellenden Ker in both years. Again, these markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Four markers associated with Pol have been mapped to sugarcane and sorghum linkage groups. In our present skeleton map, all 4 markers are associated with different sugarcane linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 and sorghum linkage group B (Appendix 1, Table 4). Marker M0K17-13 is on sugarcane Linkage Group 3. Marker M1K09-9 is on

sugarcane Linkage Group 21 and sorghum linkage group F. Marker M1K13-7 is on sugarcane Linkage Group 14.

Table 10 – Phenotypic value of markers associated with Pol

A: Markers associated across both sites and crops/years

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	5%	79.8	82.3	-2.5
MOK09X47	1%	80.7	79.8	0.9
MOK10X2	10%	79.2	81.4	-2.2
MOSSCIR172E2	6%	79.2	81.0	-1.8
M1K13X7	2%	80.2	79.3	-0.9
M1K17X28	7%	81.0	79.1	-1.9
M1PSB103E1	5%	79.0	80.6	1.6
M1SUSI2E3	1%	79.8	80.4	0.6
M2K09-115	1%	80.3	78.8	1.5
M2MO5-102	2%	79.9	81.1	-1.2

B: Markers associated with single sites (B=Bellinden Ker; M=Meringa)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
M1AB07-23	** M	84.7	81.6	-3.1
M0K05-2	*** B	75.9	78.2	-2.3
M1K17-25	**** B	76.1	78.4	2.3

D. Moisture

Moisture measurements are in g per kg. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 670.58-732.28, with a mean value of 698.3 (Table 5).

From Table 7, 8 markers were found to be significantly associated with Moisture over locations and crops/years and these are listed in Table 11A. Five markers explained 6-11% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 5-10 g/kg difference. Some markers were positively associated with moisture content while others were negatively associated.

Four additional markers were strongly correlated with Moisture at either Meringa or Bellenden Ker in both years. Again, these markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Seven markers associated with Moisture have been mapped to sugarcane and sorghum linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 and sorghum linkage group B (Appendix 1, Table 4) as are M0SSCIR51-E-2 M0AB07-7 and M0SSCIR257-H-1. Thus, these 4 markers may be detecting the same genomic region or this linkage group may contain more than one QTL for

this trait. M1K13-7 maps to sugarcane linkage group 14, while M1CDSB032-E-2 maps to sugarcane linkage group 20 and sorghum linkage group G or F. Thus, these markers are detecting at least 2 different genomic regions.

Table 11 – Phenotypic value of markers associated with Moisture

A: Markers associated across sites and crops/years.

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	11%	700.8	690.7	10.1
MOK10X2	7%	701.7	696.2	5.5
MOK17-13	9%	702.9	697.0	5.9
M1K13X7	3%	698.9	702.4	3.5
M1K17X28	6%	696.9	701.9	5.0
M1PSB103E1	11%	704.0	696.8	-7.2
M1SUSI2E3	1%	700.8	698.4	-2.4
M2MO5-102	5%	700.6	695.4	5.2

B: Markers associated with single sites (B=Bellinden Ker; M=Meringa)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
M1CDSB032-E-2	*** M	693.5	685.6	-7.9
M0SSCIR51-E-2	*** B	715.1	706.3	8.4
M0AB07-7	**** B	707.7	715.5	-7.8
M0SSCIR257-H-1	** B	707.4	715.7	-8.1

E. Fibre

Fibre measurements are in g per kg. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 98.43-156.6, with a mean value of 121.8 (Table 5).

From Table 7, 11 markers were found to be significantly associated with Fibre over locations and crops/years and these are listed in Table 12A. Three markers explained 5-16% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 4.5-7.9 g/kg difference. Some markers were positively associated with fibre content while others were negatively associated.

Four additional markers were strongly correlated with Fibre at either Meringa or Bellenden Ker in both years. Again, these markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Five markers associated with Fibre have been mapped to sugarcane and sorghum linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 and sorghum linkage group B (Appendix 1, Table 4) as does marker M0AB07-7, suggesting that these markers are detecting the same genomic region or that the linkage group contains more than one QTL for this trait. Marker M0K17-13 maps to sugarcane linkage group 3. Marker M1K09-9 maps to sugarcane

linkage group 21 and sorghum linkage group F. Marker M1K13-7 maps to sugarcane linkage group 14.

Table 12 – Phenotypic value of markers associated with Fibre

A: Markers associated with Fibre across both sites and crops/years

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	7%	119.8	127.7	-7.9
MOK09X47	1%	120.7	122.9	-2.2
MOK10X2	1%	120.3	122.4	-2.1
MOK17-13	16%	116.6	124.3	-7.7
MOSSCIR172E2	2%	122.1	119.4	2.5
M1K09-9	1%	122.6	120.4	-2.2
M1K13X7	2%	121.1	118.8	-2.3
M1K17X28	1%	122.3	120.3	-2.0
M1PSB103E1	5%	118.4	122.9	4.5
M1SUSI2E3	1%	119.8	121.7	1.9
M2MO5-102	3%	120.1	124.3	-4.2

B: Markers associated with Fibre across single sites (B=Bellinden Ker; M=Meringa)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
M0AB07-7	** ALL	124.0	118.6	5.4
M0K05-8	*** B	114.1	111.1	3.0
M1AB07-101	**** M	130.8	122.8	-8.0
M1K13-103	*** B	113.9	105.7	-8.2

F. Stalk Number

Stalk number per stool was measured for each clone. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 3.86-16.31, with a mean value of 9.1 (Table 5).

From Table 7, 11 markers were found to be significantly associated with Fibre over locations and crops/years and these are listed in Table 13A. Four markers explained 7-17% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 1.2-2.3 difference in stalk number. Some markers were positively associated with stalk number while others were negatively associated.

Two additional markers were strongly correlated with Stalk Number at Bellenden Ker in both years (Table 13B). Again, these markers may be site specific, or may be an artefact of the small population size. They will be validated on the larger Q1 population and in Q2 as part of CTA049.

Four markers associated with Stalk Number have been mapped to sugarcane and sorghum linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 and sorghum linkage group B (Appendix 1, Table 4) while M1SSCIR51-D-1 is

on Linkage Group 30. However, SSCIR51 also maps to linkage group 1, suggesting that Linkage Groups 1 and 30 belong to the same homology group. Thus, these 2 markers may be detecting the same genomic region or that this linkage group contains more than one QTL for this trait. Marker M1K09-9 maps to sugarcane linkage group 21 while marker M1K13-7 maps to sugarcane linkage group 14.

Table 13 – Phenotypic value of markers associated with Stalk Number

A: Markers associated with Stalk Number across both sites and crops/years.

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	1%	8.83	8.30	0.53
MOK09X47	6%	9.28	8.05	1.23
MOK10X2	3%	9.10	8.31	0.69
MOSSCIR172E2	1%	8.61	9.02	-0.41
M1K09-9	17%	7.61	9.44	1.83
M1K13X7	16%	10.35	8.34	-2.01
M1K17X28	2%	8.39	8.97	0.58
M1PSB103E1	14%	7.64	9.51	1.87
M1SUSI2E3	1%	8.50	9.03	0.53
M2K09-115	7%	8.9	6.6	2.3
M2MO5-102	1%	8.8	8.4	0.4

B: Markers associated with single sites (B=Bellinden Ker)

Marker	Correlation	Progeny A Mean	Progeny B Mean	Difference
MOAB04-2	**** B	9.44	6.73	2.71
M1SSCIR51-D-1	*** B	10.04	8.05	-1.99

G. Sucker Number

Sucker numbers per stool were measured for individual clones. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 1.5-8.83, with a mean value of 5.1 (Table 5).

From Table 7, 9 markers were found to be significantly associated with Sucker Number over locations and crops/years and these are listed in Table 14A. Three markers explained 8-20% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 0.8-1.25 difference in sucker number. Some markers were positively associated with sucker number while others were negatively associated.

Three markers associated with Suckering have been mapped to 3 different sugarcane linkage groups. Marker M0K10-2 is on sugarcane Linkage Group 1 and sorghum linkage group B (Appendix 1, Table 4) while Marker M1K09-9 maps to sugarcane linkage group 21 and marker M1K13-7 maps to sugarcane linkage group 14.

Table 14 – Phenotypic value of markers associated with Sucker Number

A: Markers associated with sucker number across both sites and crops/years

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOAB07x2	1%	5.16	4.71	0.45
MOK09X47	8%	5.37	4.55	0.83
MOK10X2	3%	5.29	4.79	0.50
MOSSCIR172E2	2%	4.87	5.27	-0.40
M1K09-9	17%	4.32	5.49	1.17
M1K13X7	3%	5.56	4.98	-0.58
M1K17X28	1%	4.85	5.17	0.32
M1PSB103E1	20%	4.37	5.62	1.25
M2K09-115	4%	5.1	4.1	1.0

There were no markers only significant at one or other site over both years.

H. Stem Wax

Stem wax was measured subjectively, using a 1-5 scale. Clonal means for the Q1 population from the combined analyses of variance over locations and crops/years (BSS138) ranged from 1.56-4.19, with a mean value of 3.2 (Table 5).

From Table 7, 6 markers were found to be significantly associated with Stem Wax over locations and crops/years and these are listed in Table 15A. Two markers explained 9% of the variation in this trait in the Q1 population. This level of variation is associated with approximately a 0.21-0.25 difference in stem wax rating. Some markers were positively associated with stem wax while others were negatively associated.

Two markers associated with Stem Wax have been mapped to 2 different sugarcane linkage groups. Marker M1K09-9 maps to sugarcane linkage group 21 and marker M1K13-7 maps to sugarcane linkage group 14.

Table 15 – Phenotypic value of markers associated with Stem Wax

A: Markers associated with Stem Wax across both sites and crops/years.

Marker	R square	Progeny A Mean	Progeny B Mean	Difference
MOSSCIR172E2	2%	3.21	3.30	-0.09
M1K09-9	1%	3.22	3.29	0.07
M1K13X7	3%	3.31	3.18	-0.13
M1PSB103E1	4%	3.16	3.31	0.15
M1SUSI2E3	9%	3.13	3.34	0.21
M2MO5-102	9%	3.31	3.06	0.25

There were no markers only significant at one or other site over both years.

I.Markers affecting multiple traits.

As can be seen in Table 6 and subsequent Tables, many of the markers mentioned here are significantly associated with more than one trait. However, given the significant correlation between traits (Table 6), this is to be expected. In all cases, the phenotypic effect of the marker was consistent with the positive or negative association between the traits.

Marker M0AB07-2, from 74C42, across both sites and crops, was associated with a negative effect on Brix (5.0 g/kg), CCS (3.5 g/kg), Pol (2.5 degrees Z), Fibre (7.9 g/kg) and a positive effect on moisture (10.1g/kg). This marker explained the most variation in moisture content (11%). Given the strong positive correlation between CCS, Pol, and Brix and their negative correlation with moisture, the above observation is not unexpected. There is also a strong negative association between moisture and fibre, but little correlation between the sugar-related traits and fibre.

Marker K09-47, from 74C42, across both sites and crops, was associated with an increase in stalk number (1.2) and suckering (0.8) but had little effect on the other traits. This marker explained intermediate levels of variation in stalk number and suckering (6 and 8%, respectively). Again, this result is not unexpected, given the strong positive association between stalk number and association.

Marker M0k10-2, from 74C42, across both sites and crops, was associated with a negative effect on Brix (4.2 g/kg), CCS (3.7 g.kg), Pol (2.2 g/kg) and a positive effect on moisture (5.4 g/kg). This marker was also associated with minor effects on fibre, stalk number and suckering. As mentioned above, given the strong associations between the traits, this observation is not surprising.

Marker M0K17-13, from 74C42, across both sites and crops, was associated with a negative effect on fibre (7.8 g/kg) and a positive effect on moisture (5.9 g/kg). This marker was associated with the largest amount of variation for fibre (16%).

Marker M0SSCIR172-E-2, from 74C42, across both sites and crops, was associated with a negative effect on Brix (3.3g/kg), CCS (3.9 g/kg) and Pol (1.8 g/kg), a slight negative effect on stalk number (0.4) and a slight positive effect on fibre (2.7 g/kg).

Marker M1K09-9, from Q117, across both sites and crops, was associated with an increase in stalk number (1.8) and suckering (1.2).

Marker M1K13-7, from Q117, across both sites and crops, was associated with a decrease in stalk number (2.1), a minor negative effect on suckering (0.6) and stem wax (0.13) and a minor positive effect on moisture content (3.4 g/kg)

Marker M1K17-28, from Q117, across both sites and crops, was associated with a negative effect on Brix (4.1 g/kg), CCS (3.4g/kg) and Pol (1.9 g/kg) and a positive effect on moisture (5.0 g/kg).

Marker M1PSB103-E-1, from Q117, across both sites and crops, was associated with a positive effect on Brix (4.2 g/kg), CCS (2.1 g/kg) and Pol (2.6 g/kg), an increase in fibre (4.5 g/kg), stalk number (1.9) and suckering (1.2) and a negative effect on moisture (7.1 g/kg).

Marker M1SUSI2-E3, from Q117, across both sites and crops, was associated with a positive effect on stem wax (0.21).

Marker M2K09-115, present in both parents, across both sites and crops, was associated with a positive effect of 2.3 stalks.

Marker M2MO5-102, present in both parents, across both sites and crops, was associated with an increase in moisture content (5.1 g/kg), a decrease in fibre (4.2 g/kg) and an increase in stem wax (0.25).

Comparative mapping between sugarcane and sorghum for the suckering trait.

As discussed above, by using the RFLP probes as anchors, we have been able to partially align our co-segregation groups with the sugarcane maps from TAMU and CIRAD.

We have identified several markers which are significantly associated with suckering (Tables 7 and 14, Appendix 1). We have been able to locate these onto the sorghum map of Dr Andrew Paterson and to our interest, have found that they co-locate to sorghum genomic regions associated with rhizomatousness and tillering of sorghum (Figure 1). This result strongly supports the use of sorghum as a reference map, to simplify mapping in sugarcane.

One marker, PSB103E, were strongly linked to suckering, stalk number, moisture, Brix, Pol and CCS, illustrating the strong negative relationship between the first three and latter three traits. This marker was linked to a 1.25 difference in sucker number, explaining almost 20% of the variation in suckering in the population, a 1.9 difference in stalk number and was associated with a loss of 0.2 of a CCS unit, 0.4 of Brix and 2 degrees of Pol. These preliminary analyses suggest that there are several useful regions that could be used to select against suckering. These regions will be validated as part of CTA049 by screening more progeny of this cross to obtain more reliable estimates of the value of these markers and by screening a second smaller population.

5. To isolate repetitive specific sequences for *S. officinarum* and *S. spontaneum* and use to "tag" and identify specific sugarcane chromosomes.

Small genomic libraries were made using DNA from *Erianthus arundinaceus* and Q117, as described in BSCS1. These libraries were screened with labelled total genomic DNA and several strongly hybridising clones identified. These

clones, in turn, were labelled and hybridised to membranes containing restricted genomic DNA or various *Erianthus* and *Saccharum* species. Several of these clones exhibited preferential hybridisation to one or more species. In BSCS1, several *Erianthus*-specific clones are described. Other clones were *Saccharum*-specific.

At about this point, I attended the 1997 ICSB meeting in San Diego and listened to an interesting presentation by a PhD student from CIRAD. She, too, was interested in obtaining repetitive sequences from both *Erianthus* and *Saccharum* species and had already isolated several from each genus. The same meeting also included interesting presentations from Dr Rod Wing's laboratory on the status of the sugarcane BAC library and BACs in general, and from Dr David Stelly's laboratory on *in situ* hybridisation with BACs as a means of chromosomal identification and linking physical and genetic maps. It was clear that this area of work was already further advanced at CIRAD and, in addition, *in situ* hybridisation with BACs was a much simpler and cleaner way of identifying specific chromosomes and linking physical and genetic maps. Given this information and the difficulty in mapping in our sugarcane cross, we therefore approached SRDC about expanding our mapping effort to include more individuals and dropping the repetitive sequence-related objectives from our project. This was agreed to.

6. **To locate these sequences on the genetic linkage map and associate specific chromosomes with specific linkage groups.**

See above.

IMPACT ON AUSTRALIAN SUGAR INDUSTRY, INCLUDING COST AND POTENTIAL BENEFIT

This project was established primarily as a model project to evaluate the feasibility of tagging traits of varying complexity in sugarcane with molecular markers. As such, many of the traits evaluated are traits for which molecular markers are not required and for which molecular markers are unlikely to be used, such as CCS. Notwithstanding the original objectives of the project, a number of useful findings have resulted from the project.

Firstly, we have demonstrated the feasibility of mapping in a variety x variety cross in sugarcane. We have used many common markers and can link parts of our map with other sugarcane maps, thus enabling QTL locations to be compared. More work is required in this area to fully utilise this capability, to enable additional QTLs for traits of interest, etc., to be identified.

We have also identified markers linked to major QTLs for suckering and demonstrated their strong negative effect on sucrose content. These 2 QTLs explain a significant proportion of the variation for suckering in this population. Evaluation of the effect of selection against these regions on suckering and other traits should be a priority research activity.

PROJECT TECHNOLOGY

Nil

TECHNICAL SUMMARY

See above

RECOMMENDATIONS

Numerous recommendations arise from this project.

1. It is possible to tag traits in sugarcane in variety x variety crosses. We have demonstrated that markers identified in other sugarcane crosses are useful starting points for identifying markers for those traits in Australian material. We have also demonstrated that we can identify our own markers for these and other traits not tagged in other sugarcane populations. However, this project was preliminary in terms of numbers of markers evaluated, and the number of progeny evaluated.

Recommendation:

- More progeny should be evaluated to confirm and assess more accurately the value or these markers.
- More markers are required to explain more of the variation associated with each trait, and to provide more reference points to better enable us to access information in other sorghum and sugarcane maps.
- These markers also need to be assessed in a different population, to see if these regions are generally involved in expression of these traits.
- New populations should be evaluated to identify regions not differing in the first population studied. This will allow a greater percentage of the variation in the trait to be explained.
- Some of these points will be addressed in CTA049.
- Given the high degree of inter-relationship between sugarcane varieties, a pedigree based approach to marker validation and identification would have benefit. Based on previous work in sorghum by 2 of the co-workers (Jordan and McIntyre), we have demonstrated that this approach can assist in providing additional evidence for the location of a gene for a trait in a particular QTL and in suggesting whether a particular QTL will be present in other sugarcane material.

2. The traits evaluated in this project, with the possible exception of suckering, were "model" traits. With this preliminary project we have been able to identify major QTLs and to explain a large proportion of the variation in the traits evaluated. It should now be possible to address target traits, such as disease resistance.

Recommendation:

- Initiate development of new populations for assessment of disease

- Initiate screening of existing populations for variation in disease response.
- Access markers from other laboratories which have been shown to be linked to disease traits.
- Some of these points will be addressed in CTA049.

PUBLICATIONS

1. McIntyre, C.L. and Grof, C.P.L. 1998. Sugarcane biotechnology – where are we and where to from here? In: Larkin PJ (ed.) *Agricultural Biotehcnology: Laboratory, Field and Market.* Proc. 4th Asia-Pacific Conference on Agricultural Biotechnology, Darwin 13-16 July 1998. Canberra, UTC Publishing. Pp140-143.
2. McIntyre CL, Jordan DR, Casu RE, Mortison MV and Berding N. 1999. Mapping in sugarcane – a progress report. Proc. 11th Australian Plant Breeding Conference, Adelaide 19-23 April 1999, Manning Printers. Vol 2. Pp 75-76.
3. Jordan DR, Casu RE, Besse P, Berding N, and McIntyre CL. Identification and verification of QTLs for sugar-related traits in two variety x variety crosses in sugarcane. In prep.
4. Jordan DR, Casu RE, Besse P, Berding N and McIntyre CL. Identification of major QTLs for suckering in sugarcane. In prep.