IDENTIFICATION OF DNA MARKERS LINKED TO AGRONOMIC TRAITS IN SUGARCANE IN AUSTRALIA

By

LYNNE McINTYRE1, KAREN AITKEN1, NILS BERDING2, ROSANNE CASU1, JANNEKE DRENT1, PHILLIP JACKSON1, DAVID JORDAN1,5, GEORGE PIPERIDIS3, NATHALIE REFFAY4, GRANT SMITH2, YUEZHI TAO1,5 and VICKI WHAN1

1CSIRO Plant Industry, 120 Meiers Rd, Indooroopilly, Qld 4068, Australia
2Bureau of Sugar Experiment Stations, PO Box 122, Gordonvale, Qld 4865
3Bureau of Sugar Experiment Stations, 50 Meiers Rd, Indooroopilly, Qld 4068
4Universite de la Reunion, St Denis, La Reunion, France
5QDPI Hermitage Research Station, Warwick, Qld 4370

Abstract

In Australia, molecular maps are being constructed in several sugarcane populations, to identify markers linked to agronomic traits of interest including sugar-related traits, fibre, suckering, and resistance to diseases such as common rust, orange rust, Pachymetra, smut, yellow spot and Fiji Disease. Markers are also being developed to facilitate introgression of new genes from S. officinarum germplasm into elite sugarcane clones. This paper briefly describes our current research and interests in these areas.

Introduction

The production of new cultivars of sugarcane using conventional breeding techniques takes many years and is complicated by sugarcane's complex genetic structure. Molecular marker technology has great potential to contribute to the genetic improvement of sugarcane by identifying novel genetic variation, improving the heritability, and expediting the production of elite genetic material, as well as providing an improved understanding of the sugarcane genome. In Australia, molecular maps are being constructed in several sugarcane and sorghum populations, to identify markers linked to priority traits, including sugar-related traits, fibre, suckering, and resistance to diseases such as common rust, orange rust, Pachymetra, smut, yellow spot and Fiji Disease. Markers are also being developed to facilitate introgression of new genes from S. officinarum germplasm into elite sugarcane clones to broaden the narrow genetic base of sugarcane.

Sugarcane, sorghum and maize are members of the Andropogonae tribe. Comparative mapping between these 3 species has revealed a high level of synteny (Hulbert et al., 1990; Grivet et al., 1994; Dufour et al., 1997). In particular, sorghum and sugarcane appear to be more closely related than either is to maize (Grivet et al., 1997). Sorghum is a diploid species with a very small genome (2n = 20; 760 Mbp/1C) (Arunuganathan and Earle, 1991) while chromosome numbers in Saccharum species range from 2n = 36-170 and the DNA content is 3-6 times larger (2547-4183 Mbp/1C) and similar to the maize genome size. This suggests that parallel studies on sugarcane and the simpler and more densely mapped sorghum genome will facilitate the mapping of the more complex sugarcane genome.

Materials and methods (more details are available on request)

Plant material

Three sugarcane populations are currently being evaluated.

Population1: The Q117 x 74C42 population of 312 clones was made by Nils Berding (BSES, Gordonvale, Australia). The population has been evaluated for 8 sugar-related and morphological traits—CCS, brix, pol, fibre, moisture, stalk number, suckering and stem wax—using standard BSES protocols. Data have also been collected for resistance to common rust, and to Pachymetra, orange rust and yellow spot.

Population 2: The Q117 x MQ77-340 population of 230 clones was made by Phillip Jackson (CSIRO). It is currently being evaluated for sucrose and fibre content.

Population 3: I76-514 x Q165 population of 238 clones was made by George Piperidis (BSES) and Phillip Jackson (CSIRO). This officinarum x variety cross will be evaluated for sucrose and fibre content.

Three sorghum populations have been developed by Bob Henzell (QDPI, Warwick). These populations have been phenotyped for drought tolerance, yield, midge resistance, maturity, height, rust resistance and bacterial leaf blight. Disease screening for resistance to ergot, smut and Fusarium is underway.

Molecular markers

DNA was isolated and RFLP analysis performed using the methods described in the Laboratory Protocols Manual developed by CIMMYT (Hoisington, DA). Laboratory Protocols. CIMMYT Applied Molecular Genetics Laboratory, Mexico, D.F.). RFLP probes were obtained from Angélique D'Hont, CIRAD

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(SSCIR clones) and from Andrew Paterson, UG (sugar metabolism probe set). Microsatellite sequences and protocols were obtained from the International Consortium of Sugarcane Biotechnology (Cordeiro et al., 2000). All techniques relevant to the RAF (Radio-labelled amplified fingerprint) were as developed by Bernie Carroll (UQ). AFLP were performed using a BRL kit.

**Linkage analysis**

All progeny were checked to ensure that they are progeny of the respective parents of the cross. Markers which segregate 1:1 are identified and used for linkage analysis using MapQTL (Van Ooijen and Maliepaard, 1996).

**Results and Discussion**

**Genetic mapping in Q117 × 74C42**

To date, we have scored approximately 400 markers on 120 progeny of the population. These markers comprised RFLP, RAF, SSR and AFLP markers. Single factor analysis has been undertaken with most of the markers generated to date. Linkage has been found between markers for all of the sugar, fibre and morphological traits measured. Twelve markers have been found that are significantly associated with one or more of these traits across both years and both sites. These 'robust' markers explained varying percentages of the variation in a trait in the population and were both positively and negatively associated with each trait (Table 1).

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. markers explaining &gt;5% variation across both sites and years</th>
<th>Maximum % variation explained by a marker for each trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brix</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>Pol</td>
<td>5</td>
<td>10%</td>
</tr>
<tr>
<td>CCS</td>
<td>3</td>
<td>7%</td>
</tr>
<tr>
<td>Moisture</td>
<td>6</td>
<td>11%</td>
</tr>
<tr>
<td>Fibre</td>
<td>3</td>
<td>16%</td>
</tr>
<tr>
<td>Stalk Number</td>
<td>5</td>
<td>17%</td>
</tr>
<tr>
<td>Suckering</td>
<td>3</td>
<td>20%</td>
</tr>
<tr>
<td>Stem Wax</td>
<td>2</td>
<td>9%</td>
</tr>
</tbody>
</table>

In general, only small amounts of variation in the sugar-related traits of up to 10% 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. com.). For brix, 3 markers with effects of 8–10% were identified. For pol, 5 markers each explained more than 5% of the variation while, for CCS, 3 markers explained between 5 and 7% of the variation. Markers strongly associated with one trait were usually strongly associated with the other 2 traits. Five robust markers were found to be associated with moisture content and explained 6–11% of the variation. For fibre, three markers were identified which explained 5-16% of the variation. For stalk number, 4 markers explaining 7-17% of the variation were identified, while for suckering, 3 markers explaining between 8 and 20% of the variation were found. Two markers each explaining 9% of the variation were found for stem wax. Many of the markers were significantly associated with more than one trait. Given the obvious correlation between traits, this is not surprising. For example, markers associated with a positive effect on moisture, were usually associated with a negative effect on sugar-related traits and on fibre, and vice-versa. Several markers associated with stalk number were also (but not always) associated with suckering.

Our current and future efforts are focussed on mapping more SSRs from the ICSB (Cordeiro et al., 2000) and AFLP markers to enable alignment of this map with other sugarcane maps (Grivet et al., 1996; Ming et al., 1998, Hoarau et al., 2001) and to identify markers linked to disease resistance. Field data are currently being collected on resistance to common rust, Pachymetra, orange rust, yellow spot and Fiji Disease. Pachymetra and Fiji Disease are major pathogens of Australian sugarcane and are very difficult, time consuming and expensive to screen for. As such, they are prime targets for molecular markers. Other disease screening, including leaf scald and red rot, is also planned.

We have also initiated a candidate gene mapping approach. Sugarcane ESTs (Casu et al., 2001; ISSCT paper) from categories relevant to the traits segregating in this population have been identified and approximately 400 have been screened over the parents of the mapping population. We will soon begin progeny screening and mapping with these genes to see if any map to QTLs of interest. These genes will be further investigated as perfect markers in sugarcane breeding. This approach may also provide additional confirmation of the role of these genes in the sugarcane plant. We are increasingly using sorghum as a reference map (see below), given the excellent synteny between the two crops and, consequently, as many as possible of our sugarcane markers are concurrently mapped in sorghum. This provides additional markers for our sorghum analyses and greatly simplifies mapping in sugarcane. More than 100 sugarcane ESTs have been mapped in sorghum.

**Genetic mapping in Q117 × MQ77-340**

Mapping in this cross has only just begun. This cross is known to segregate widely for sucrose content, more widely than the cross above, and thus should enable more sugar-related QTLs to be mapped. Our mapping strategy will be to use SSRs which have already been mapped onto our maps, AFLPs to provide marker numbers, and candidate genes for the sugar and fibre biosynthetic pathways. The use of SSRs and candidate genes should allow this map to be aligned with other maps produced here and overseas.

**Genetic mapping in LJ 76-514 × Q165**

The 239 progeny of this cross have been screened with 4 RAF primers, 5 AFLP primers and 30 SSR
primers, generating approximately 400 markers to date. Our goal is to get to approximately 800 markers by May when the first sucrose content data on the progeny will be available. Progeny will be selected on the basis of high sucrose content and presence of desirable QTLs, especially those which derive from *S. officinarum*. These progeny will be grown out and crossed with elite sugarcane clones in 2 years time. At this time, progeny again will be identified using markers, which have as many desirable QTLs as possible and as few IJ76-514-specific markers as possible. The progeny will also be phenotyped to verify the results obtained by molecular markers. Our introgression studies are described more fully in the paper by Albertson *et al.* (2001).

**Comparative mapping with sorghum**

Wherever possible, markers are mapped simultaneously onto one of our 3 sorghum mapping populations. Two of these populations have between 300 and 500 markers on them but polymorphism levels are only about 20%. DPI has recently developed an interspecific sorghum cross with polymorphism levels of approximately 50%. We are now using this population as our reference population. The 3 sorghum populations are extensively linked by sorghum microsatellites and common RFLPs. The sorghum and sugarcane populations are primarily linked via RFLP probes in common, but also by some sugarcane microsatellites which map to sorghum.

**REFERENCES**


