

GENETIC MAPS OF *Saccharum officinarum* L. AND *Saccharum robustum* BRANDES & JEW. EX GRASSL *

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ABSTRACT

Genetic analysis was performed in a population composed of 100 F₁ individuals derived from a cross between a cultivated sugarcane (*S. officinarum* 'LA Purple') and its proposed progenitor species (*S. robustum* 'Mol 5829'). Various types (arbitrarily primed-PCR, RFLPs, and AFLPs) of single-dose DNA markers (SDMs) were used to construct genetic linkage maps for both species. The LA Purple map was composed of 341 SDMs, spanning 74 linkage groups and 1,881 cM, while the Mol 5829 map contained 301 SDMs, spanning 65 linkage groups and 1,189 cM. Transmission genetics in these two species showed incomplete polysomy based on the detection of 15% of SDMs linked in repulsion in LA Purple and 13% of these in Mol 5829. Because of this incomplete polysomy, multiple-dose markers could not be mapped for lack of a genetic model for their segregation. Due to inclusion of RFLP anchor probes, conserved in related species, the resulting maps will serve as useful tools for breeding, ecology, evolution, and molecular biology studies within the Andropogoneae.

INTRODUCTION

Saccharum L. is part of a polyploid complex within the Andropogoneae tribe. Cultivated forms of *Saccharum* (sugarcane) are most notably used for sugar and alcohol production worldwide, especially in the tropics. Sugarcane is the most genetically complex crop for which genome mapping has been achieved (Al-Janabi *et al.*, 1993; daSilva *et al.*, 1995). Polyploidy in *Saccharum* is widespread and is largely responsible for its genetic and taxonomic complexity. Studies using DNA markers and molecular cytogenetics revealed polysomic inheritance and octoploidy ($x = 8$) within *S. spontaneum* ($2n = 64$, from India) (Al-Janabi *et al.*, 1993; daSilva *et al.*, 1995; D'Hont *et al.*, 1996). The basic chromosome number and level of ploidy have not been conclusively determined for other *Saccharum* species.

Due to its genetic peculiarities, molecular genetic markers cannot be applied to sugarcane as they are to most plants. Use of DNA markers has recently allowed genetic mapping in polyploids (daSilva and Sobral, 1996). A novel genetic approach to direct mapping of polyploid plants was proposed by Wu *et al.* (1992). This approach is based on single-dose markers (SDMs). SDMs are present in one parent, absent in the other parent, and segregate 1:1 in the

progeny. More recently, daSilva (1993) and Ripol (1994) presented a methodology for mapping multiple dose markers in polysomic polyploids, which greatly improved the accuracy of identification of homology groups (daSilva *et al.*, 1993, 1995).

Restriction fragment length polymorphisms (RFLPs) were the first DNA markers used to construct genetic maps of higher organisms (Botstein *et al.*, 1980). DNA fingerprinting methods, based on amplification of random genomic DNA fragments by arbitrarily selected primers (Welsh and McClelland, 1990; Williams *et al.*, 1990), have also been used for genetic mapping (Al-Janabi *et al.*, 1993) among other applications (Welsh *et al.*, 1991). More recently, amplified fragment length polymorphisms (AFLPs), a technique based on selective PCR amplification of genomic restriction fragments, have provided another very powerful tool for genomic research (Vos *et al.*, 1995). When mapping with single-dose polymorphisms, all bands are scored as dominant markers, therefore the typical advantage of RFLPs, namely codominance of markers, is lacking. Thus, PCR-generated markers with an inherently higher data output per unit labor are good choices for generating and saturating linkage maps (Sobral and Honeycutt, 1993; Vos *et al.*, 1995). However, RFLPs remain the most informative marker to determine homologous relationships among chromosomes within *Saccharum* and among grasses, including maize and sorghum.

S. officinarum is a domesticated species, which is thought to have been derived primarily from *S. robustum*, a wild species in Papua New Guinea (Brandes, 1929). We herein report the development of SDM linkage maps for each of these species using RFLP- and PCR-based markers for progeny of an interspecific cross. These maps have also been used in comparative studies among sugarcane, sorghum and maize, and in the analysis of quantitative traits in these two species (Guimarães *et al.*, 1997).

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MATERIAL AND METHODS

Plant materials

Plant materials were kindly provided by the Hawaiian Sugar Planters' Association (Aiea, HI). The population consisted of 100 F₁ individuals produced by crossing *S. officinarum* 'LA Purple' as female with *S. robustum* 'Mol 5829'. Cytological evaluation of the population showed that parents and progeny displayed strict bivalent pairing at meiosis and had $2n = 80$ chromosomes, as described previously by Al-Janabi *et al.* (1994a).

DNA markers

RFLPs

Genomic DNA was extracted according to the method of Honeycutt *et al.* (1992). Fifteen µg of genomic DNA from parents and 100 progeny was restricted individually with *Dra*I, *Eco*RI, *Hind*III, and *Xba*I, and resolved in agarose gels. The gels were blotted and Southern hybridization was performed according to daSilva (1993). After hybridization, blots were exposed to BioMax film (Kodak) at -80°C for 3 to 7 days depending on signal intensity. One hundred and ninety probes were surveyed against parental DNA blots digested individually with the four enzymes to identify scorable polymorphisms. Subsequently, probes were hybridized to genomic DNA blots of the F₁ population that had been digested with the appropriate enzyme.

Heterologous maize genomic clones (UMC - University of Missouri-Columbia, and BNL - Brookhaven Natl. Laboratory) and maize cDNAs (ISU - Iowa State University) previously mapped in maize and sorghum were used as RFLP probes. Sugarcane genomic DNA (SG) clones and cDNA clones from buds (CSB), cell culture (CSC), and roots (CSR), which were previously mapped in *Saccharum spontaneum* 'SES 208' (daSilva *et al.*, 1993, 1995), were also used as RFLP probes. Cloned genes from sucrose metabolism and transport pathways, including *smpl-1*, a sugarcane membrane protein and putative glucose transporter (Bugos and Thom, 1993), *sps-1*, sucrose phosphate synthase from maize (Worrell *et al.*, 1991), *SS-1*, maize sucrose synthase (McCarty *et al.*, 1986), and *HBr-1*, a maize phosphoglucosyltransferase-encoding probe (kindly provided by S. Briggs, Pioneer Hi-Bred International, Johnston IA), were also used as RFLP probes.

Arbitrarily primed PCR

Genomic DNAs from parents and progeny (25 ng) served as templates for thermal cycling in a System Cycler 9600 (Perkin Elmer), using the protocol described by Sobral and Honeycutt (1993). Arbitrarily primed PCR products were resolved on either agarose or polyacrylamide gels. Agarose gel electrophoresis and recording and

scoring of amplified products were performed according to Al-Janabi *et al.* (1993). Arbitrarily primed PCR products amplified with α^{32} P-dCTP were resolved in 5% polyacrylamide-50% urea gels in 1x Tris-borate-EDTA, and visualized by autoradiography using BioMax film (Kodak) at room temperature for 1-3 days. Over 400 ten-mers of arbitrary sequence (Operon Technologies, Inc.) and four RY-repeat twelve-mers (CG6 - 5'-TCGCTGCGGCGG-3', CG7 - 5'-CTGCGGTGCGG-3', CG8 - 5'-CAGCCGTAGCGG-3', and CG9 - 5'-CCGCGACTGCGG-3') were screened against the mapping parents.

Selective restriction fragment amplification

AFLPs (Vos *et al.*, 1995) were generated using AFLP Analysis System I (Gibco-BRL). Two hundred and fifty ng of genomic DNA from parents and progeny was simultaneously digested to completion with *Eco*RI and *Mse*I. Restricted genomic DNA fragments were ligated to *Eco*RI and *Mse*I adapters, diluted 1:10, and pre-amplified using AFLP core primers, each having one selective nucleotide. Pre-amplification products were then diluted to 1:50 and used as a template for selective amplification using the combinations of *Mse*I- and *Eco*RI-specific primers, each containing three selective nucleotides. *Eco*RI-selective primers were labeled with γ^{32} P-ATP before amplification. The thermal profile for both steps of amplification, primer labeling, and selective primer combinations were performed as recommended by the manufacturer. The selective amplified products were resolved by electrophoresis in denaturing polyacrylamide gel, as described for arbitrarily primed PCR.

Marker identification

RFLPs were named by using the original probes' identification (*umc*, *bnl*, *isu*, *csb*, *csc*, *csr* or *sg*), followed by the first letter of the restriction enzyme used (d, e, h, or x for *Dra*I, *Eco*RI, *Hind*III, or *Xba*I, respectively), followed by a period and the molecular size (in base pairs). Size was a single-gel estimate calculated by linear regression and standardization against a 1-kb ladder (Gibco, BRL) for each blot. Arbitrarily primed-PCR polymorphisms were named using the Operon denomination (from A to Z and from 1 to 20), or the RY-repeat primer designation (CG6-CG9), followed by a period and the molecular size (in base pairs). The arbitrarily primed PCR polymorphisms that are followed by the letter *p* were resolved in denaturing polyacrylamide gels, while the others were resolved in agarose gels. AFLPs were coded by the *Eco*RI (E) and *Mse*I (M) selective primer combination and the respective molecular size (in base pairs).

Linkage analysis

Polymorphisms were scored for presence (1) and absence (0), and analyzed for dosage among F₁ progeny

using chi-square tests ($P < 0.05$), as described by Wu *et al.* (1992) and daSilva *et al.* (1995). Because of the double-pseudo-testcross mating strategy used (reviewed in daSilva and Sobral, 1996), SDMs are identified in each of the parents, resulting in two maps: one for the male parent and one for the female parent. Linkage relationships among SDMs were determined using MapMaker v 2.0 for the Macintosh (Lander *et al.*, 1987) by coding the data as haploid (as the population is resultant from a double pseudo-testcross mating strategy). SDMs were grouped using a minimum LOD of 7.0 and a maximum recombination fraction (r) of $r < 0.25$ (Wu *et al.*, 1992). Linkage groups were then ordered using multi-point analyses. Markers at $r < 3$ cM could not be ordered accurately because of the relatively small sample size; however, the best possible order was always accepted, even if the LOD score supporting the order was not large. Map distance in centimorgans was calculated using the Kosambi mapping function. Linkages in repulsion phase were determined as described by Al-Janabi *et al.* (1993).

RESULTS AND DISCUSSION

Linkage maps

A total of 341 single-dose DNA markers were mapped in the LA Purple genome, yielding 74 linkage groups (Figure 1) with 58 unlinked SDMs. In Mol 5829, linkage analysis of 301 SDMs generated 65 linkage groups (Figure 2), while 93 SDMs remained unlinked under the chosen criteria. In LA Purple the linked markers spanned 1,881 centimorgans (cM) for an average of 6.65 cM per marker. In Mol 5829, linked markers covered 1,189 cM, an average of 5.74 cM per marker.

Marker distribution and mapping output

The total number of polymorphisms between the two genomes analyzed was significantly different with $\chi^2 = 4.49$; $P < 0.05$ (503 for LA Purple and 438 for Mol 5829)

(Table I). *S. officinarum* showed a higher level of polymorphism for all marker types. Sixty-eight percent of the polymorphisms generated were single-dose. This percentage is similar to the results of daSilva (1993), that found 73% of polymorphisms in *S. spontaneum* to be single dose.

Markers generated by different methods were not uniformly distributed across the linkage groups. In LA Purple, 26% of the linkage groups had all three marker types, and in Mol 5829, just 9% of the linkage groups were covered by all types of markers. Lack of uniform distribution may be accounted for simply by the different numbers of each type of marker mapped on each genome (Table I) and the incomplete saturation of both genomes with markers. daSilva *et al.* (1995) mapped 208 AP-PCR markers and 234 RFLPs in *S. spontaneum* 'SES 208', and they did not find significant deviation from a random distribution of the markers among linkage groups.

Of the 190 maize probes surveyed against the parental sugarcane DNA, 131 probes produced a good hybridization pattern. The signal produced with maize genomic and cDNA probes suggests a high degree of DNA sequence similarity among these species, despite at least 25 million years of evolution since they shared a common ancestor (Al-Janabi *et al.*, 1994b; Sobral *et al.*, 1994). A similar result was reported by daSilva *et al.* (1993), in which 78% of maize probes surveyed produced a strong signal in *S. spontaneum*.

Chromosome assortment in *S. officinarum* and *S. robustum*

Both repulsion and coupling phase linkages were observed in *S. officinarum* and *S. robustum* genomes. Fifteen percent of LA Purple markers were detected in repulsion phase and were assigned to 17 linkage groups having at least one repulsion phase SDMs. Similarly, 13% of Mol 5829 were in repulsion phase and were assigned to 11 linkage groups.

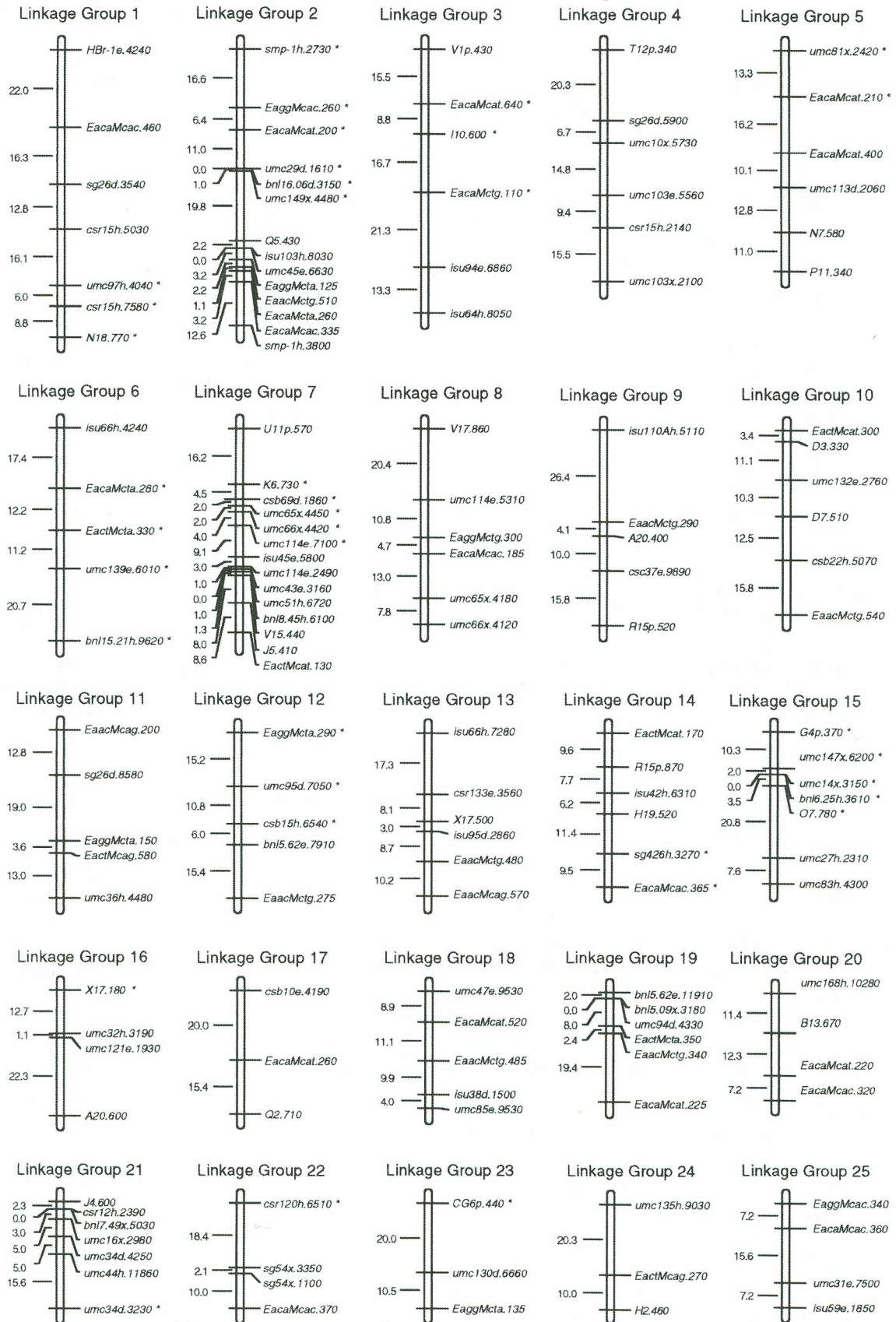
If complete preferential pairing of homologous chromosomes (as in diploids and disomic polyploids) were observed in these species, then linkages in both repulsion

Table I
Summary of marker data.

	RFLP			AP-PCR			AFLP			All markers		
	LAP	Mol	Total	LAP	Mol	Total	LAP	Mol	Total	LAP	Mol	Total
Number of experiments ^a			166			64			12			
Number of polymorphisms	271	268	539	126	78	204	135	107	242	532	453	985
Number of SDMs ^b	172	173	345	73	54	127	96	74	170	341	301	642
SDMs linked	151	129	280	55	31	86	77	47	124	283	207	490
Total polymorphisms/exp.			3.2			3.2			20.2			
SDMs/exp.			2.1			2.0			14.2			

^aEach experiment consists of: RFLP, probe/restriction enzyme combination; AP-PCR, primer reaction; AFLP, *EcoRI* and *MseI* selective primer combination. ^bSingle-dose markers (SDMs) determined using a χ^2 test ($P < 0.05$), as described by Wu *et al.* (1992). LAP, *Saccharum officinarum* LA Purple; Mol, *Saccharum robustum* Mol 5829.

Figure 1 - Genetic linkage map of *Saccharum officinarum* 'LA Purple'. Seventy-four linkage groups were detected with an LOD = 7 and $r = 0.25$ for two-point analysis. The numbers to the left of the linkage groups represent the genetic distance in centimorgans as calculated by using the Kosambi function. The markers are shown on the right of the linkage groups; markers followed by an asterisk (*) are linked in repulsion phase.



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Figure 1 Continued

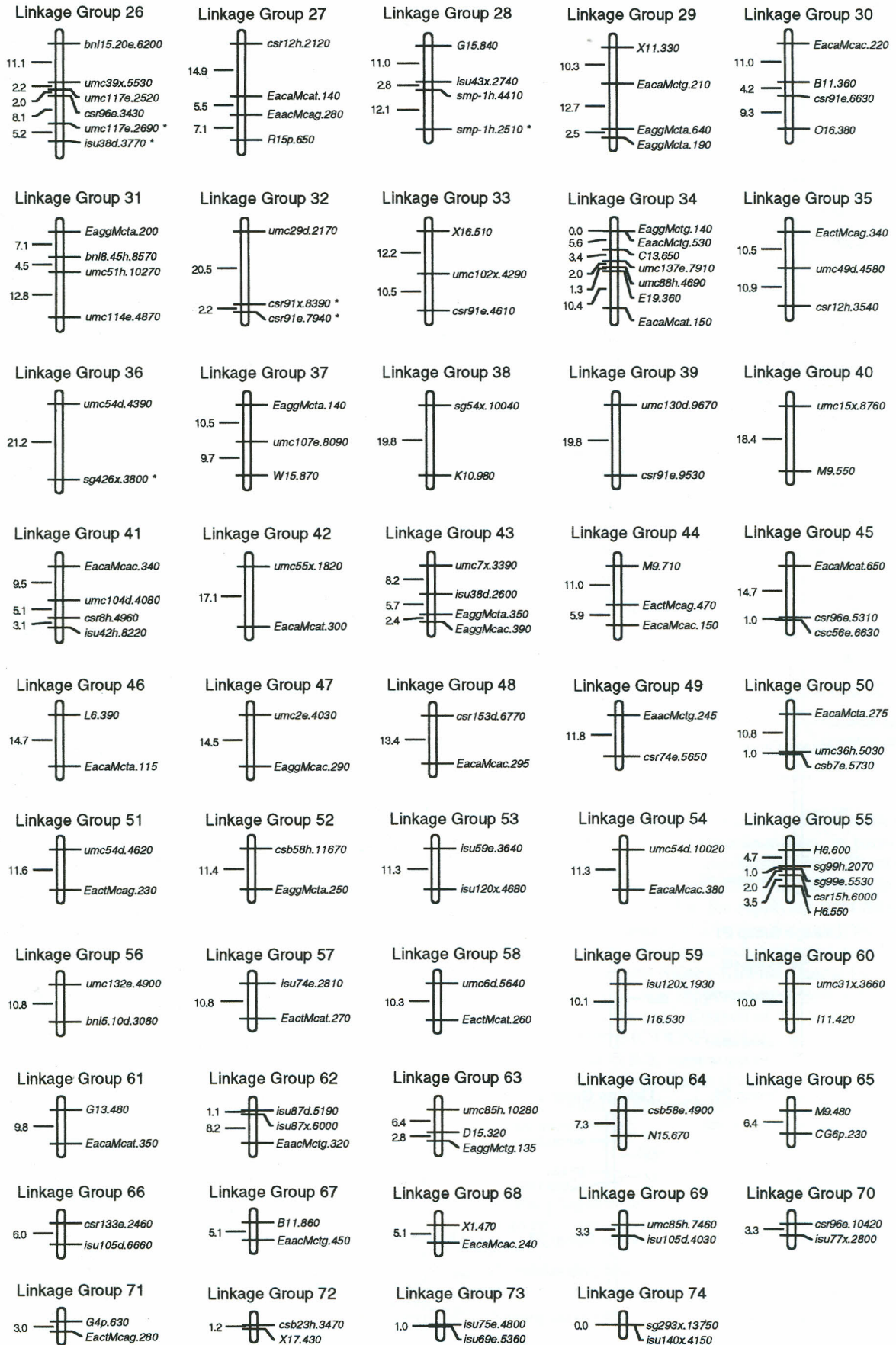
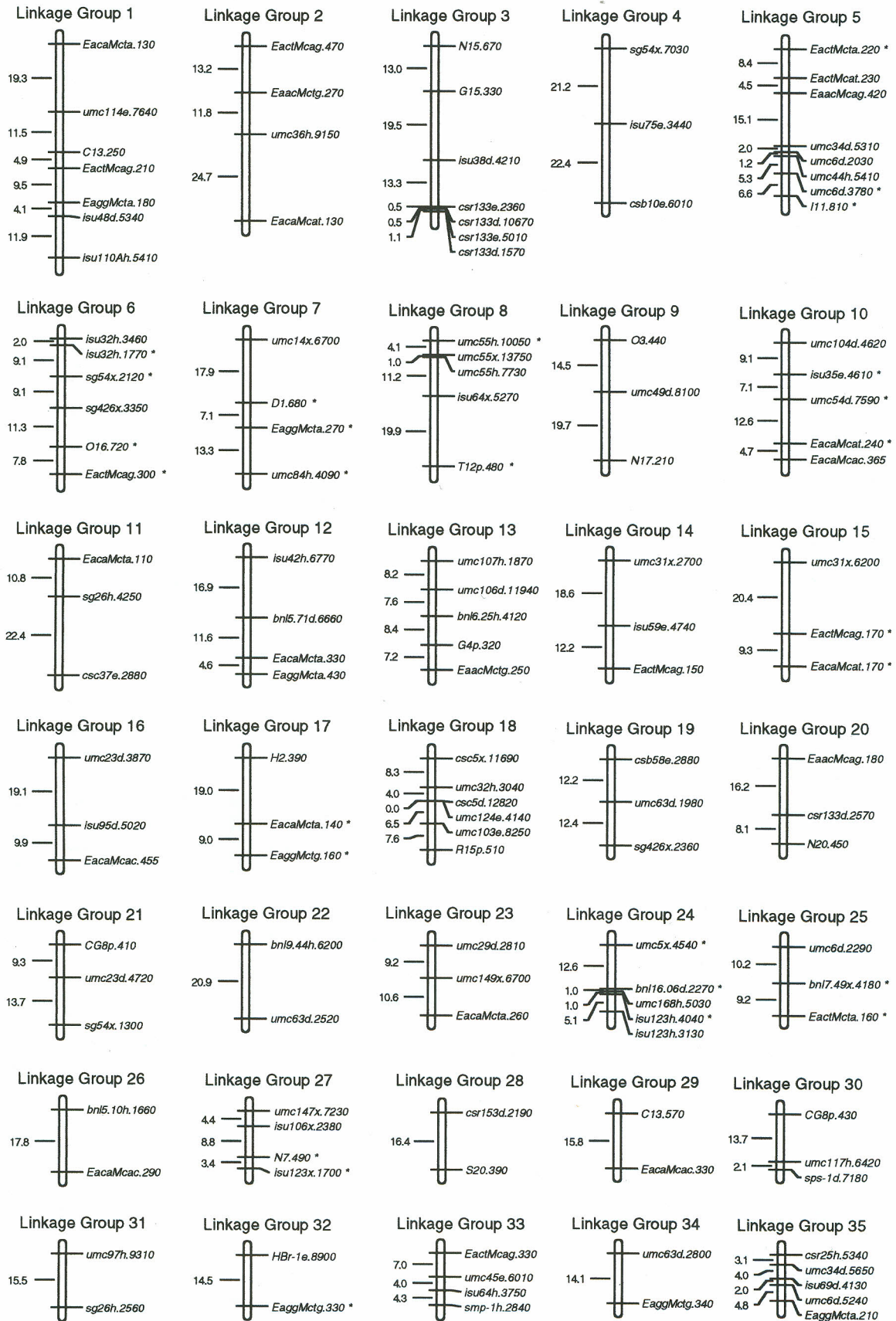
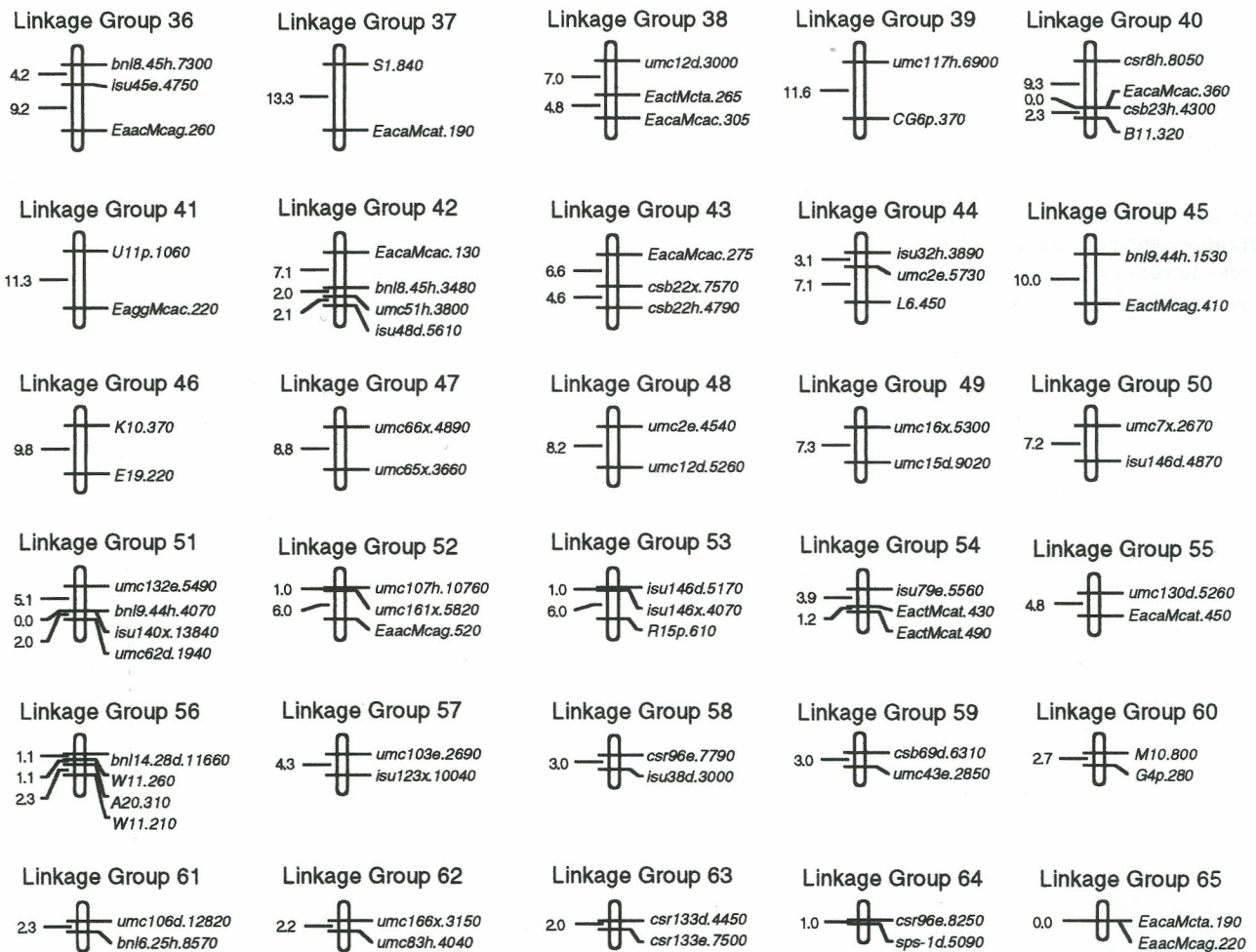


Figure 2 - Genetic linkage map of *Saccharum robustum* 'Mol 5829'. Sixty-five linkage groups were detected with an LOD = 7 and $r = 0.25$ for two-point analysis. The numbers to the left of the linkage groups represent the genetic distance in centimorgans as calculated by using the Kosambi function. Markers are shown on the right of the linkage groups; markers followed by an asterisk (*) are linked in repulsion phase.



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Figure 2 Continued



and coupling phases should occur at approximately equal frequencies. If chromosome pairing were completely random, as in polysomic polyploids, then markers linked in repulsion phase at a maximum of 10 cM would require a mapping population of at least 750 individuals for their detection, assuming a polysomic octaploid with strict bivalent pairing (Wu *et al.*, 1992). Detection of repulsion phase linkages in both genomes with a population size of only 100 individuals strongly suggests that these genomes are neither fully polysomic nor disomic. This result agrees with a previous study of a subset of 44 individuals from this population (Al-Janabi *et al.*, 1994a) and with Mudge *et al.* (1996), who studied the same cross with arbitrarily primed PCR markers. This strongly suggests preferential chromosome pairing, at least for some linkage groups. Linkages in repulsion imply that a saturated map for euploid *S. officinarum* or *S. robustum* will have less than $2n = 80$ linkage groups.

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RESUMO

Uma progênie de 100 indivíduos F_1 obtidos de um cruzamento entre cana-de-açúcar (*S. officinarum* 'LA Purple') e seu suposto progenitor (*S. robustum* 'Mol 5829') foi analisada utilizando marcadores moleculares em dose única. Marcadores do tipo AP-PCR, RFLP e AFLP, gerando um total de 642 polimorfismos, foram mapeados em ambas espécies. O mapa genético de LA Purple foi composto de 341 marcadores, distribuídos em 74 grupos de

ligação e 1.881 cM, enquanto que o mapa de ligação de Mol 5829 continha 301 marcadores ao longo de 65 grupos de ligação e 1.189 cM. A transmissão genética nessas duas espécies apresentou polissomia incompleta devido a detecção de 15% dos marcadores em dose simples ligados em fase de repulsão e 13% desses em Mol 5829. Devido a essa polissomia incompleta, os marcadores em dose múltipla não puderam ser mapeados por falta de um modelo genético para descrever tal segregação. O mapeamento de sondas de RFLP, conservadas entre espécies próximas evolutivamente, permitirá que os mapas genéticos gerados sejam utilizados como poderosas ferramentas no melhoramento e em estudos de ecologia, evolução e biologia molecular dentro das Andropogoneae.

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