ORIGINAL PAPER

A. A. F. Garcia · E. A. Kido · A. N. Meza
H. M. B. Souza · L. R. Pinto · M. M. Pastina
C. S. Leite · J. A. G. da Silva · E. C. Ulian
A. Figueira · A. P. Souza

Development of an integrated genetic map of a sugarcane (*Saccharum* spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases

Abstract Sugarcane (*Saccharum* spp.) is a clonally propagated outcrossing polyploid crop of great importance in tropical agriculture. Up to now, all sugarcane genetic maps had been developed using either full-sib progenies derived from interspecific crosses or from selfing, both approaches not directly adopted in conventional breeding. We have developed a single integrated genetic map using a population derived from a cross between two pre-commercial cultivars ('SP80-180' × 'SP80-4966') using a novel approach based on the simultaneous maximum-likelihood estimation of linkage and linkage phases method specially designed for outcrossing species. From a total of 1,118 single-dose markers (RFLP, SSR and AFLP) identified, 39% derived from a testcross configuration between the parents segregating in a 1:1 fashion, while 61% segregated 3:1, representing heterozygous markers in both parents with the same genotypes. The markers segregating 3:1 were used to establish linkage between the testcross markers. The final map comprised of 357 linked markers, including 57 RFLPs, 64 SSRs and 236 AFLPs that were assigned to 131 co-segregation groups, considering a LOD score of 5, and a recombination fraction of 37.5 cM with map distances estimated by Kosambi function. The co-segregation groups represented a total map length of 2,602.4 cM, with a marker density of 7.3 cM. When the same data were analyzed using Join-Map software, only 217 linked markers were assigned to 98 co-segregation groups, spanning 1,340 cM, with a marker density of 6.2 cM. The maximum-likelihood approach reduced the number of unlinked markers to 761 (68.0%), compared to 901 (80.5%) using JoinMap. All the co-segregation groups obtained using JoinMap were present in the map constructed based on the maximum-likelihood method. Differences on the marker order within the co-segregation groups were observed between the two maps. Based on RFLP and SSR markers, 42 of the 131 co-segregation groups were assembled into 12 putative homology groups. Overall, the simultaneous maximum-likelihood estimation of linkage and linkage phases was more efficient than the method used by JoinMap to generate an integrated genetic map of sugarcane.

Keywords Linkage map · AFLP · SSR · RFLP · Polyploid

E.A. Kido, A.N. Meza and H.M.B. Souza contributed equally to this work.

A. A. F. Garcia (✉) · M. M. Pastina
Departamento de Genética, Escola Superior
de Agricultura Luiz de Queiroz (ESALQ), Universidade de São
Paulo (USP), CP 83, 13400-970 Piracicaba-SP, Brazil
E-mail: aafgarci@esalq.usp.br

E. A. Kido · H. M. B. Souza · A. Figueira
Centro de Energia Nuclear na Agricultura,
Universidade de São Paulo, Av. Centenário, 303,
CP 96, Piracicaba, SP 13400-970, Brazil

A. N. Meza · L. R. Pinto · C. S. Leite · A. P. Souza
Centro de Biologia Molecular e Engenharia Genética (CBMEG),
Universidade Estadual de Campinas (UNICAMP),
Cidade Universitária "Zeferino Vaz", CP 6010,
13083-970 Campinas-SP, Brazil

J. A. G. da Silva
Texas Agricultural Experiment Station, Weslaco Center,
Texas A and M University, 2415 E. Hwy. 83, Weslaco,
TX 78596, USA

E. C. Ulian
Copersucar Technology Center, Caixa Postal 162,
13400-970 Piracicaba, São Paulo, Brazil

Introduction

Sugarcane (*Saccharum* spp.) is a crop with major economic importance in many tropical countries. Modern sugarcane cultivars are highly polyploid with chromosome numbers in somatic cells (2*n*) ranging from 100 to

130. These cultivars derived from interspecific hybridization between *Saccharum officinarum* L. ($2n = 70$–140) and *Saccharum spontaneum* L. ($2n = 36$–128) (Irvine 1999). Cultivars are vegetatively propagated, and result from selection in populations obtained from crosses between outcrossing heterozygous parents.

The development of genetic linkage maps in polyploids became feasible with segregation analysis of single-dose markers, proposed by Wu et al. (1992). Single-dose markers represent an allele present in one copy (simplex allele) in only one of the two parents, displaying a 1:1 segregation ratio in the progeny, as in a testcross configuration. Since all the genotypes cannot be easily identified by their banding phenotypes, this mapping strategy adopted for polyploid species, with bivalent pairing at meiosis, permits segregation analysis of multiple alleles at several dosages (Wu et al. 1992). Markers in single-dose present in both parents, with an expected 3:1 segregation ratio can also be used for genetic mapping, but they are less informative.

Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) or microsatellites are molecular markers currently used in sugarcane genetic mapping (Mudge et al. 1996; Hoarau et al. 2001; Rossi et al. 2003) and in genetic diversity studies (Lima et al. 2002). Basically, sugarcane linkage maps had been developed for progenies derived from crosses involving wild relatives (*S. spontaneum, S. officinarum*, and *Saccharum robustum*) of cultivated sugarcane (D'Hont et al. 1994; Mudge et al. 1996; Ming et al. 1998; Guimarães et al. 1999). The first published linkage map was derived from a cross between 'SES 208' (*S. spontaneum*) and its double haploid 'ADP 85-0068' using RFLP (Da Silva et al. 1993) and RAPD (Al-Janabi et al. 1993) single-dose markers that were later combined in a single map (Da Silva et al. 1995). More recently, a genetic map based on AFLP markers, estimated to cover one-third of the genome length was developed from a self-progeny of the sugarcane elite cultivar R570 (Hoarau et al. 2001) previously mapped with RFLP markers (Grivet et al. 1996).

The double pseudo-testcross strategy, in which single-dose polymorphisms identified in each parent allow the construction of two individual specific maps, is a common approach used to map outcrossing species (Grattapaglia and Sederoff 1994; Shepherd et al. 2003; Porceddu et al. 2002; Carlier et al. 2004). Genetic linkage maps for *S. officinarum* ('LA Purple') and *S. robustum* ('Mol 5829') were constructed using RAPD, RFLP and AFLP single-dose markers based on a double-pseudo-testcross strategy (Guimarães et al. 1999). However, the integration of such parental individual maps into a single map can only be done if markers heterozygous in both parents ("intercross markers") are used to establish relationships between markers segregating in each parent ("testcross markers") (Barreneche et al. 1998; Wu et al. 2000).

An integrated map comprising of different types of molecular markers (RFLPs, RAPDs, SSRs and AFLPs) should bring several advantages such as increasing saturation, extending the characterization of polymorphic variation throughout the entire genome. Specifically for polyploids, co-dominant markers can be helpful to assemble co-segregation groups into their respective homologous groups (Da Silva et al. 1993; D'Hont et al. 1994; Grivet et al. 1996). In addition, the location of quantitative trait loci (QTLs) is facilitated if an integrated map is available (Maliepaard et al. 1998). However, in heterozygous parents, for each segregating loci, there can exist different numbers of segregating alleles or markers. This situation complicates linkage analysis and mapping once parental linkage phases of marker pairs are unknown a priori, which makes the detection of recombination events difficult (Maliepaard et al. 1997; Wu et al. 2002).

The genetic complexity of sugarcane makes mapping a challenging task. Many statistical methods and software are available for genetic linkage analysis, such as MAPMAKER/EXP (Lander et al. 1987) and JoinMap (Van Ooijen and Voorrips 2001). JoinMap was specially designed to deal with full-sib mapping population, and implemented the statistical methods described by Maliepaard et al. (1997). More recently, Wu et al. (2002) proposed a novel approach that applies the maximum-likelihood method for the simultaneous estimation of linkage and linkage phases in outcrossing species, resolving several of the difficulties presented by Maliepaard et al. (1997) for the estimation of linkage phases. This method seems promising for the construction of an integrated genetic map for sugarcane, combining different amounts of information generated by various types of molecular markers. The method proposed by Wu et al. (2002) can be applied to any species that requires the use of a full-sib progeny (F_1 generation) derived from outbred parents as a mapping population.

The present study is the first to report the construction of an integrated genetic map derived from a cross between two sugarcane elite pre-commercial cultivars selected from the Cane Technology Center (Piracicaba, São Paulo, Brazil) breeding program. The map combined various sources of polymorphism data (AFLP, RFLP and SSR), analyzed using the method proposed by Wu et al. (2002).

Materials and methods

Plant material

The mapping population was obtained from a cross between pre-commercial cultivars 'SP80-180' [B3337 × polycross] (lower sucrose content; high stalk production) and 'SP80-4966' [SP71-1406 × polycross] (higher sucrose; lower stalk production). The population is comprised of 498 individuals of which, 100 were randomly chosen for mapping.

DNA extraction and genotyping

Total genomic DNA was extracted from 300 mg of powdery lyophilized young leaf tissue using a CTAB-method (Hoisington et al. 1994), with minor modification for sugarcane. Three types of markers were used to genotype the parents and the 100 progeny individuals. The markers were generated as follows:

RFLP

Genomic DNA (20 μg) was individually digested with four restriction enzymes (*Dra*I, *Eco* RI, *Eco* RV and *Xba* I). Restriction fragments were separated on 0.8% (w/v) agarose gels that were run in TAE buffer (40 mM Tris acetate, pH 8.0; 2 mM EDTA) at 20 mA for 22 h and transferred to nylon membranes (Hybond-N$^+$, Amersham, UK). Probes were radioactively labeled with ^{32}PdCTP using the Rediprime II kit (Amersham). Hybridizations were performed in a HYB solution (0.5 M Na$_2$PO$_4$ pH 7.2, 1% BSA, 7% SDS, 100 μg/mL sheared herring sperm DNA) at 65°C for 18–24 h. The membranes were washed once during 20 min at 65°C in each of the following solutions: solution I (2 × SSC; 5% SDS), solution II (1 × SSC; 5% SDS 5%) and solution III (0.5 × SSC; 5% SDS) and placed on X-Omat (Kodak, Japan) film for at least 7 days at -80°C. The 83 sugarcane genomic SG probes used (Burnquist 1991) were randomly selected.

SSR

A total of 259 microsatellite loci were tested, with 183 developed by Cordeiro et al. (2000) and 76 from CIRAD (Centre de Cooperation Internationale em Recherché Agronomique pour le Développement, Montpellier, France) as described by Rossi et al. (2003) and supplied by the international consortium of sugarcane biotechnology-ICSB. The amplification reactions were performed in 25 μl final volume, containing 25 ng of template DNA; 0.2 μM of each forward and reverse primers; 100 μM of each dNTP; 1.5 mM MgCl$_2$; 10 mM Tris–HCl pH 8.8, 50 mM KCl; 0.1% Triton X-100 and 1 U *Taq* DNA polymerase (Invitrogen, São Paulo, SP, Brazil). Amplification conditions for each primer were supplied by ICSB and CIRAD. Amplification were conducted on a GeneAmp PCR System 9700 thermocycler (Applied Biosystem, Foster City, CA, USA) programmed with an initial step of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing temperature at 50 or 55°C according to primer pair for 30 s, extension at 73°C for 30 s, with a final cycle at 73°C for 3 min. The 76 CIRAD primers were amplified with an initial step at 94°C for 5 min followed by 35 cycles of 94°C for 45 s; annealing temperature at 50 or 55°C according to each primer for 30 s; extension of 73°C for 30 s; and a final elongation step at 73°C for 30 s. Amplification products were mixed in a 2:1 ratio with a denaturing loading buffer (98% formamide; 10 mM EDTA; 0.025% bromophenol blue; 0.025% xylene cyanol) and denatured at 95°C for 5 min. Samples were loaded onto denaturing 7% polyacrylamide gels and electroforesed in 1 × TBE buffer at 60 W for 1 h 45 min. Gels were silver stained according to Creste et al. (2001).

AFLP

Analysis was performed using the AFLP Analysis System I Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA (250 ng) was digested with *Eco*RI and *Mse*I, and the restricted fragments were linked to *Eco*RI and *Mse*I adapters. Pre-selective amplifications were performed with one selective nucleotide primer. Selective amplifications were made with three selective nucleotide primers, in which the *Eco*RI sites were end-labeled with γ-[^{33}P]-ATP (4.000 Ci/mmol). Amplifications were conducted on a PTC^{TM}-100 thermocycler (MJ Research, Watertown, MA). Amplification products were mixed in a 2:1 ratio with a loading buffer (as above) and denatured at 90°C for 4 min. Denatured samples (3.5 μL) were loaded on 5% denaturing polyacrylamide (20:1) gels with 7 M urea in 1 × TBE buffer using a 48-sharktooth comb. Electrophoresis was performed in a sequencing apparatus (S2001, Life Technologies) at 65 W during 2 h 15 min. Gels were dried for 1 h at 80°C and exposed to a Biomax MR (Kodak) film for 5 days at −80°C. Sixty-four primer combinations of the AFLP kit were tested using the mapping parental cultivars.

Marker notation

RFLP markers were identified with the genomic probe (SG) number, followed by a letter indicating a decrease in size of the fragment (molecular weight), and a letter to denote parental polymorphism origin. The SSR markers were named according to locus origin (SMC refers to loci developed by Cordeiro et al. (2000) and CIR refers to CIRAD) followed by a number referring to the amplified allele and a letter to denote parental polymorphism origin. The AFLP markers were labeled by the *Eco*RI and *Mse*I selective primer sequences of three letters, followed by a number indicating a decrease in size of the fragment (molecular weight), followed by a letter to denote parental polymorphism origin. Parental polymorphism origin was designated according to the cross type of the marker locus, following notation of Wu et al. (2002): "D$_1$" marker locus heterozygous in 'SP80-180' and homozygous in 'SP80-4966' with the cross-type configuration "*ao × oo*", "D$_2$" marker locus heterozygous in 'SP80-4966' and homozygous in 'SP80-180' with the cross-type configuration "*oo × ao*" and "C" marker locus heterozygous in both parents with a cross-type configuration "*ao × ao*". The single-dose allele (simplex) is symbolized by "*a*" and is dominant in relation to the null allele (nulliplex) symbolized by "*o*". The "D$_1$" and

"D$_2$" groups refer to loci that are in a testcross configuration between the parents and segregate in a 1:1 ratio (asymmetrical marker cross types), while "C" refers to heterozygous loci in both parents that segregate in a 3:1 ratio, with the same genotypes in both parents (symmetrical marker cross type). For homology groups (HGs) notation, each HG received a Roman numeral from I to XII (HGI to HGXII), followed by a number for their respective co-segregation group (CG). The unassigned CGs were numbered consecutively.

Marker segregation

Each marker was scored based on the presence (1) or absence (0) in the 100 progeny individuals of the mapping population. Unreliable markers were considered as missing data and discarded up to a limit of 25. Marker segregation types were identified in a chi-square test (χ^2) for deviation from the segregation ratios of 1:1 and 3:1, expected for markers in single-dose in only one of the parents (testcross configuration), and markers in single-dose condition in both parents (here referred to as double single-dose markers), respectively. A low type I error of 0.000045 was considered to discard loci with strong deviation from expected proportions.

Linkage analysis

First, linkage analysis and map construction were performed with JoinMap Version 3.0 (Stam 1993;

Maliepaard et al. 1997; Van Ooijen and Voorrips, 2001). Map distances expressed in centiMorgans (cM) were estimated based on the recombination fraction using the Kosambi function. Due to the large number of evaluated loci (1,118), a minimum LOD score threshold of five was adopted for linkage analysis. The JoinMap LOD score is based on the chi-square statistic for independency and differs from the LOD score usually adopted in linkage analysis, which is affected by segregation distortion (Maliepaard et al. 1998). The maximum-likelihood estimates of recombination fractions (r) between markers (loci) were obtained (Maliepaard et al. 1997), using direct formulas (linkage between markers segregating 1:1) or the EM algorithm (Dempster et al. 1977) for the remaining ones. Linkage phases were determined with the recombination fraction considering all possible linkage phases between markers (coupling–coupling; coupling–repulsion; repulsion–coupling; and repulsion–repulsion). Subsequently, deduction of the most probable linkage phases was carried out observing if the r estimate was smaller than 0.5 and if a significant LOD score was obtained (Maliepaard et al. 1997). Next, the markers were ordered and the multi-point distances were estimated (Van Ooijen and Voorrips 2001). For the ordering step, the parameters used were $r < 0.50$, $LOD > 5.00$ and jump < 5. Also, the use of three rounds was allowed to get the map, but this was not necessary in any case. Different values were tried for these parameters and no great changes in the maps were found.

In a second step, a genetic map was also constructed using the method described by Wu et al.

Table 1 Marker polymorphism used for mapping

Marker type	RFLP	SSR	AFLP	Total
Number of markers evaluated	221	355	1104	1680
Number of polymorphic markers between parents	112	190	304	606
[a]Single-dose markers (1:1)	100	129	212	441
Number of monomorphic markers between parents	109	165	800	1074
[b]Double-single-dose markers (3:1)	89	81	507	677
Total Number (1:1 and 3:1)	189	210	719	1118
Number of markers with distorted segregation	32	145	385	562

[a]Marker present in only one parent with a 1:1 segregation ratio in the mapping population
[b]Marker present in both parents with a 3:1 segregation ratio in the mapping population

Table 2 Distribution of the different marker types according to their cross type

[a]Cross type	No of markers				No of linked markers			
	RFLP	SSR	AFLP	Total[a]	RFLP[b]	SSR[b]	AFLP[b]	Total[c]
D$_1$ (ao × oo)	47	55	80	182 (16)	19 (1.7)	15 (1.3)	36 (3.2)	70 (20)
D$_2$ (oo × ao)	53	74	132	259 (23)	16 (1.4)	26 (2.3)	76 (6.8)	118 (33)
C (ao × ao)	89	81	507	677 (60)	22 (1.9)	23 (2.0)	124 (11)	169 (47)
Total	189	210	719	1118	57	64	236	357

[a]According to Wu et al. 2002. Percentages in *parenthesis*
[b]Percentage of linked markers in relation of the total number of markers available for mapping (1118)
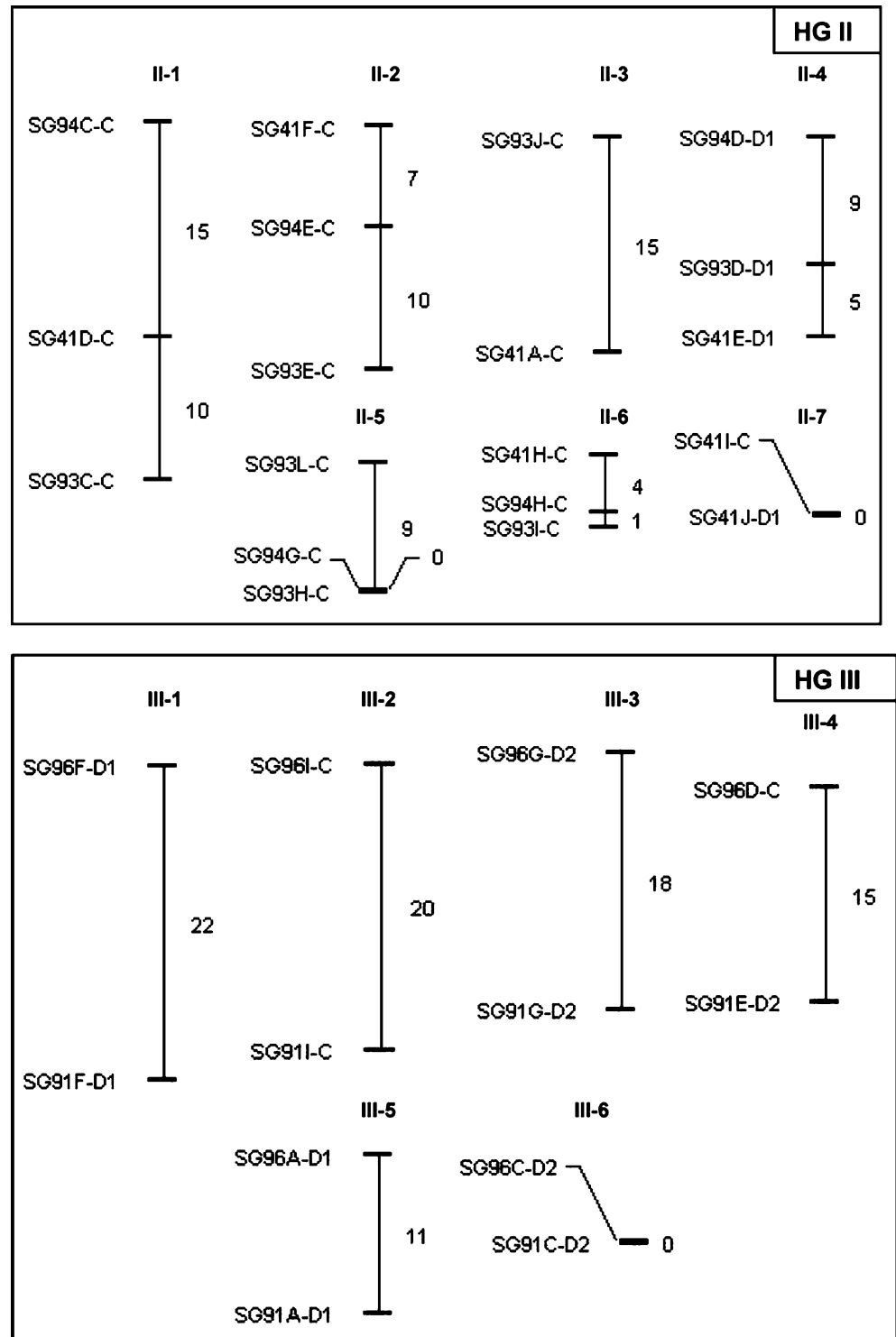[c]Percentage in relation to the total number of linked markers (357)
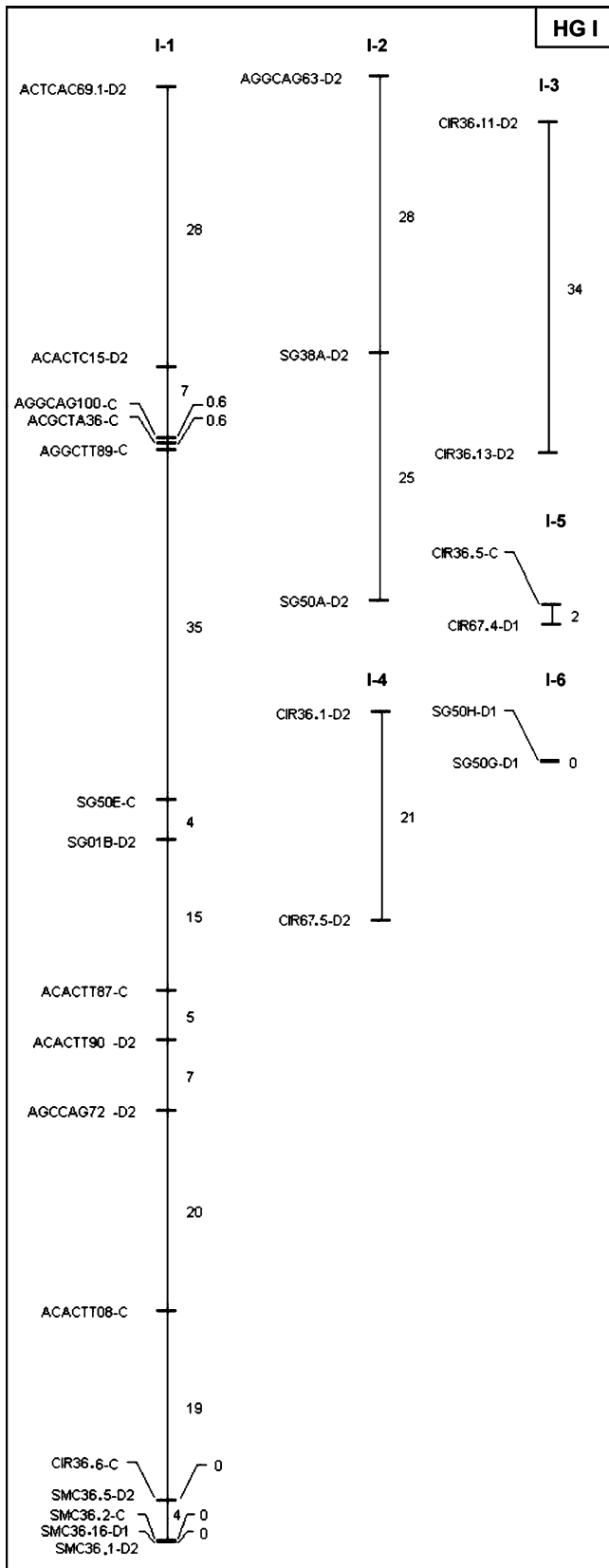
Fig. 1 Integrated genetic map of a sugarcane commercial cross (SP80-180 × SP80-4966) based on 100 F$_1$ individuals. Map distances are given in centi-Morgans (Kosambi). Homology groups (*HG*s) are assembled in a rectangle with a Roman numeral from I to XII (HGI to HGXII). Co-segregation groups are numbered inside each HG, and unassigned co-segregation groups (*CG*s) with more than two markers are numbered consecutively. The CGs with two markers are in Table 3

JoinMap were used, and a two-point analysis was applied between all possible marker pairs within each co-segregation group, estimating simultaneously the recombination fraction and the linkage phases between markers, based on posterior probabilities. Although three types of markers (D$_1$, D$_2$ and C) are available for sugarcane analysis, the D$_1$ and D$_2$ combination do not allow sufficient information to distinguish between recombinant and non-recombinant progeny individuals. Thus, their linkage relationships were deduced indirectly based on linkages of other marker types of the same co-segregation group. For the D$_1$ vs. D$_1$ or D$_2$ vs. D$_2$ markers, the recombination fraction between markers could be estimated by maximizing the log-likelihood. For the remainder of marker types, the EM algorithm was applied (Dempster et al. 1977). The linkage phase between two markers was estimated according to the posterior probability that is based on Bayes' theorem and calculated for all possible linkage phases considering both parents. Thus, the linkage phase showing the highest posterior probability was considered as the most probable to have occurred. Next, for each co-segregation group, a matrix with all recombination fractions between all possible marker pairs was constructed and used for marker ordination via *Rapid Chain Delineation* (RCD) (Doerge 1996) and *Seriation* (Buetow and Chakravarti 1987) algorithms. The marker order suggested by the algorithms was also checked with the *Ripple* algorithm (Lander et al. 1987) based on minimum sum of adjacent recombination fractions (SARF) and maximum sum of adjacent lod score (SALOD) (Liu 1998) and in the likelihood of each sub-order (Wu et al. 2002). The number of markers included on ripple varied from three (likelihood) up to six (RCD and Seriation). Comparing the results of these algorithms, after the definition of the most probable order, the genetic map was constructed showing the multi-point estimates (3-point) of the distance between markers after the application of the Kosambi function (Wu et al. 2002). To perform such an analysis, a software was developed under the *R* statistical package (R Development Core Team, 2004) (http://www.R-project.org) and it was named OneMap. (A user-friendly version of it is under development and soon will be made available; for more details, contact the corresponding author). Co-segregation groups (CGs) were assembled into putative homology groups (HGs) based on markers derived from the same SSR locus or RFLP probe.

(2002) that allows simultaneous estimation of the recombination fraction and the linkage phases. For this purpose, the co-segregation groups established by

Fig. 1 (Contd.)

Results

Segregation analysis

The three types of markers evaluated (RFLP, SSR and AFLP) generated a total of 1,118 single-dose markers. Markers present in single-dose in both parents with a 3:1 segregation ratio were also considered and referred to as double single-dose markers. Of the 1,118 markers, 61% were double single-dose markers and 39% single dose. The markers that did not fit the expected segregation ratio (1:1 for single dose or 3:1 for double single-dose) were considered to be distorted markers and/or at higher allele dosages. Overall, markers that deviated from the two expected ratios corresponded to 33% of the total

Fig. 1 (Contd.)
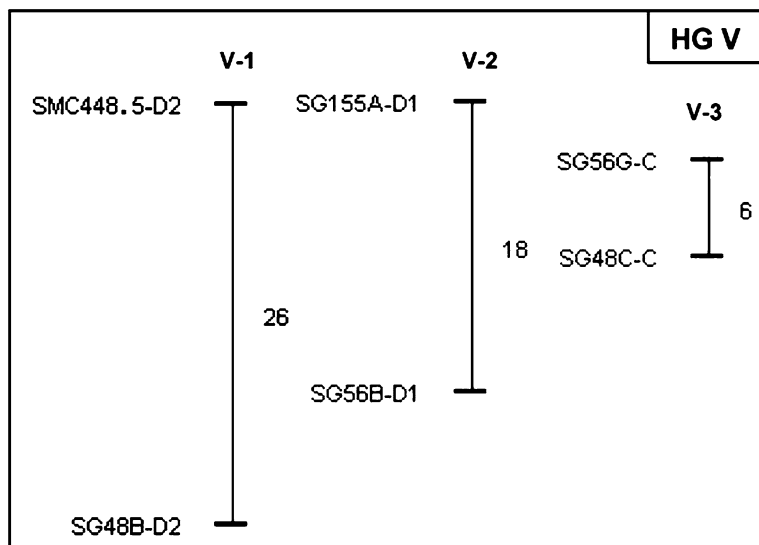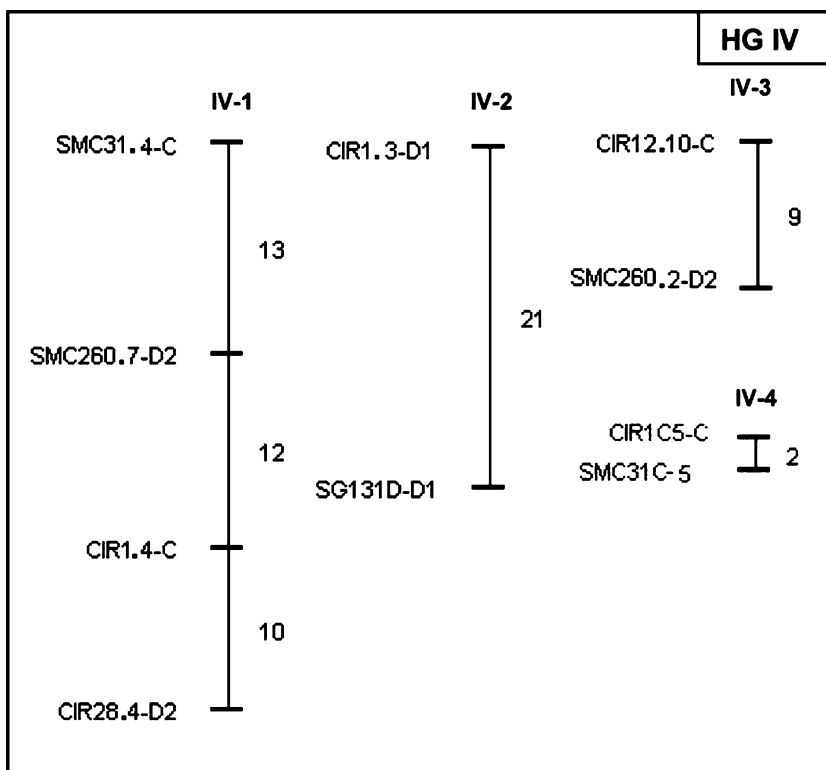
number (1,680) evaluated for segregation (Table 1). Details for each marker type are presented separately as follows:

RFLP markers

Of the 83 probe/enzyme combinations, 55 were selected according to the number, intensity and quality of the polymorphic bands. Markers displaying low-intensity signals may represent a region of low copy number, probably, in single dose. From the 55 probe/enzyme combinations, a total of 221 markers were detected, from which 112 were polymorphic between the two parents, with 100 markers (89.3%) behaving as single dose, segregating in a 1:1 ratio in the progeny. The remaining 109 markers were monomorphic between the parents, but only 89 (81.7%) segregated at a 3:1 ratio in the progeny and were assumed to be in single dose in both parents (Table 1).

SSR markers

From the 259 SSRs loci screened for the two parents, a total of 52 SSR loci (20%) were selected for mapping. These 52 SSR loci amplified 355 scorable

Fig. 1 (Contd.)
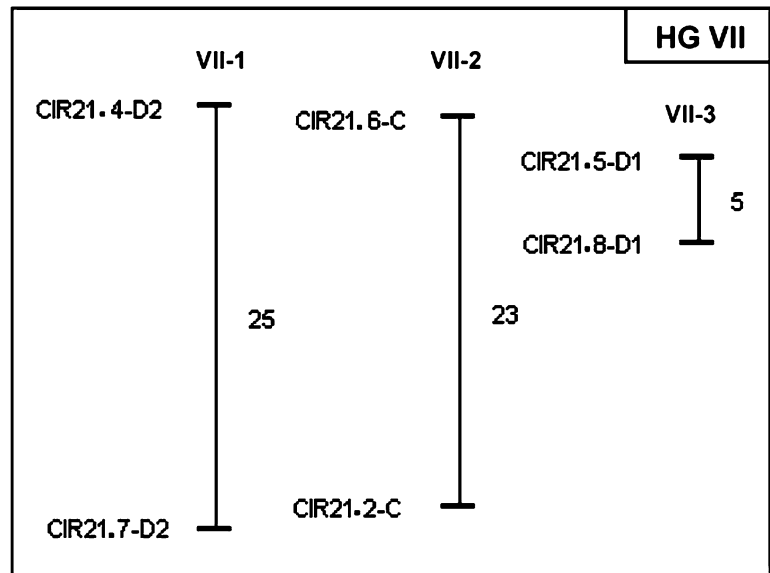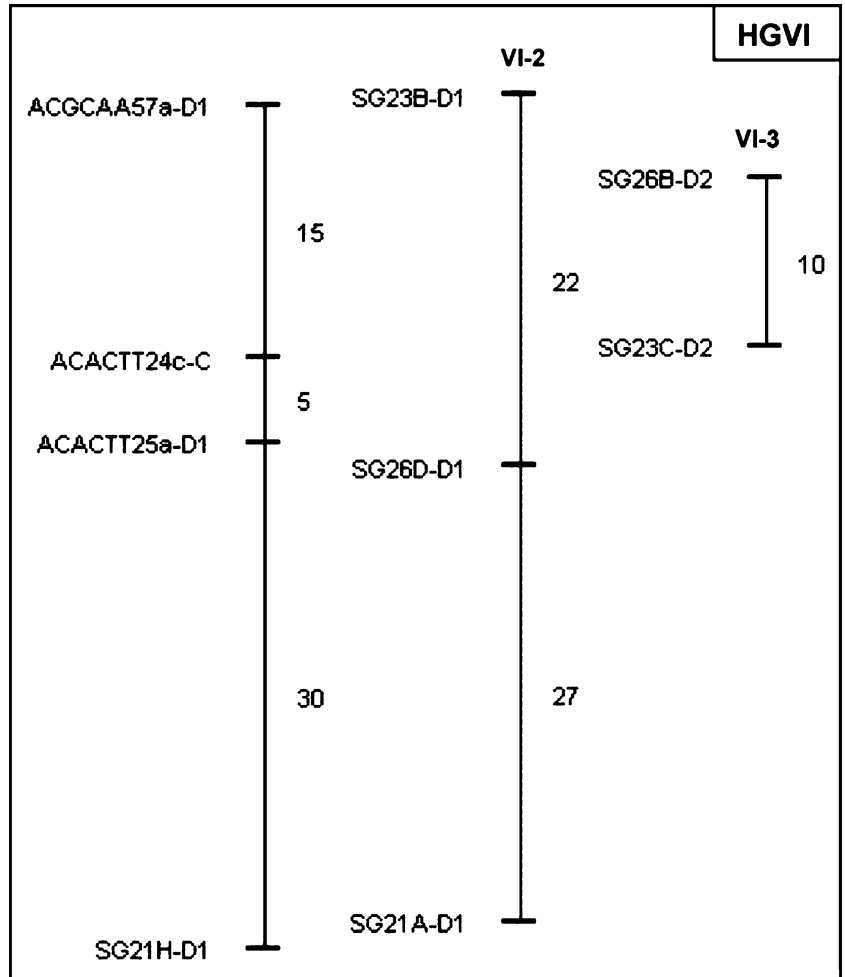
markers (alleles), with an average of 6.8 markers per locus. Out of these markers, 129 (36%) showed polymorphism between the parents with no segregation distortion from the 1:1 ratio expected for single-dose markers in the mapping population, whereas 81 markers (23%) were monomorphic between the parents, and segregated at a 3:1 ratio in the mapping population (Table 1).

Fig. 1 (Contd.)
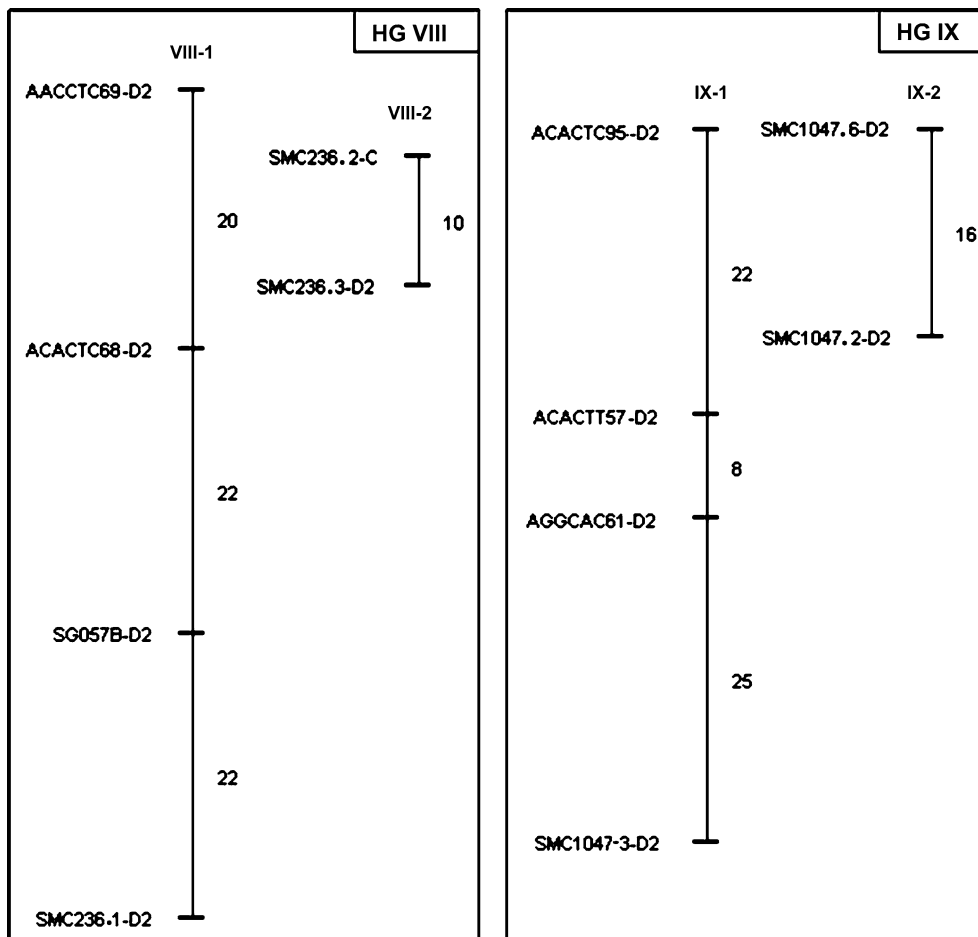
AFLP markers

From the 64 primer combinations tested, 23 were selected for mapping, producing a total of 2,502 markers (mean of 109 markers per primer combination), of which 1,770 (71%) were considered as reliable markers for segregation analysis. Among the 1,770 markers, 304 (17%) were polymorphic between the parents, 800 (45%) were monomorphic between parents but polymorphic in the mapping population. The remaining 666 (38%) markers corresponded exclusively to monomorphic markers. Thus, a total of 1,104 markers were effectively available for segregation analysis. Two hundred and twelve of the 304 markers were at single dose, whereas 507 of the 800 markers were considered to be double single-dose markers (Table 1).

Cross types

Each marker data set (RFLP, SSR and AFLP) was subdivided into three groups according to their respective cross type (Table 2). The cross-type configuration "*ao* × *ao*" (marker locus heterozygous in both parents) predominated among all marker classes (RFLP, SSR and AFLP), contributing with 47% of the total number of linked markers. For all types of markers, the number of loci with the "*oo* × *ao*" testcross configuration was higher than the "*ao* × *oo*" with higher levels of single-dose markers coming from the male parent 'SP80-4966'.

Linkage map

The linkage map obtained using JoinMap (not shown), based on the methodology developed by Maliepaard et al. (1997), comprised 217 markers distributed into 98 co-segregation groups (CG). Although JoinMap established 131 CG, only 98 CG were assembled, since the software alleged "insufficient linkage information" for the remaining co-segregation groups when the "MAP" command was requested. For ordering purposes, these groups had to be divided into smaller ones, but this was not done to avoid the loss of information in comparison with the complete ones. Using the method proposed by Wu et al. (2002), 357 (32%) markers were grouped at a LOD ≥ 5, while the other 761 (68%) remained unassigned to co-segregation groups. The mapped markers consisted of 57 RFLPs, 64 SSRs and 236 AFLPs (Table 2), and were assigned to 131 CGs (Fig. 1; Table 3). Most of the co-segregation groups (91 or 70%) were formed with two linked markers; 13 CG with three

Fig. 1 (Contd.)
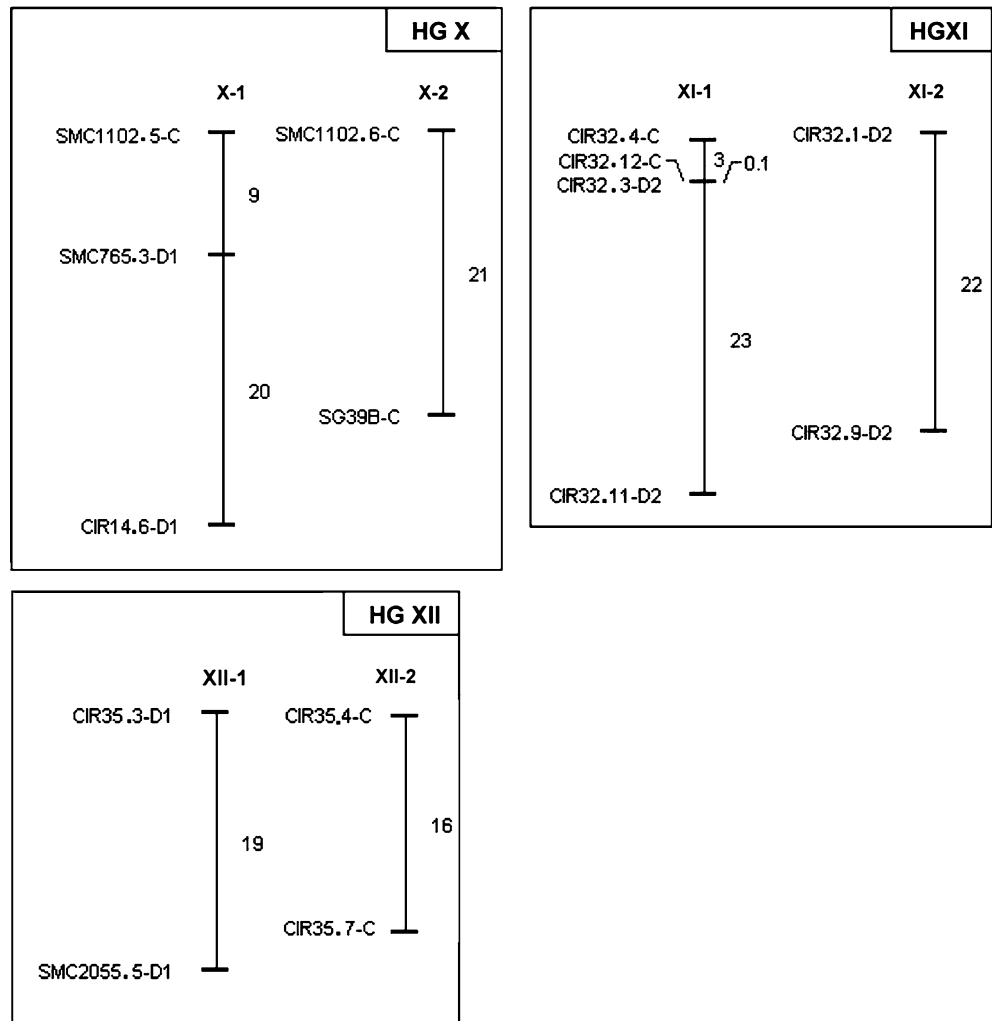
linked markers; 17 CG with four markers; three CG with five markers; two CG with six and three with eight linked markers; and one group displayed ten linked markers, while another had 16 linked markers. The map covered 2,602.4 cM, with an average distance between markers (marker density) of 7.3 cM (Table 4). Some marker loci were clustered along the CGs, while others were sparsely distributed with gaps larger than 20 cM, being observed on 30% of the intervals between two adjacent marker loci.

The number of unlinked markers was 68% smaller using the maximum-likelihood method of Wu et al. (2002) in comparison to JoinMap (Table 4). All CG obtained using JoinMap were present in the map developed based on the maximum-likelihood method, with major differences attributed to marker order. The map obtained by JoinMap covered only 1,340 cM, with a marker density of 6.2 cM (Table 4).

Homology groups

Based on the co-dominance of the RFLP and SSR markers, it was possible to assemble the CGs into homology groups (Fig. 1). From the final 131 co-segregation groups, 42 were assembled into 12 putative homology groups (HGs). The remaining groups did not contain any marker in common, standing as independent groups. The number of CG assembled in each HG varied from 2 to 7. Two homology groups (HG VII and HG XI) were formed with co-segregation groups having markers derived from only one SSR locus, respectively, CIR21 (HG VII) and CIR32 (HG XI).

Discussion

Segregation analysis and cross types

Linkage analysis with single-dose markers has been successfully adopted in mapping polyploid outcrossing crops, when inbred lines cannot be easily developed to generate backcrosses or F_2 mapping progenies (Da Silva et al. 1995, Mudge et al. 1996, Hoarau et al. 2001). Independent of ploidy level ($2n = 4X$, $6X$, $8X$ and $10X$), a progeny of 75 individuals was considered large enough to detect single-dose loci at high confidence levels (Wu et al. 1992). Our single-dose markers were identified
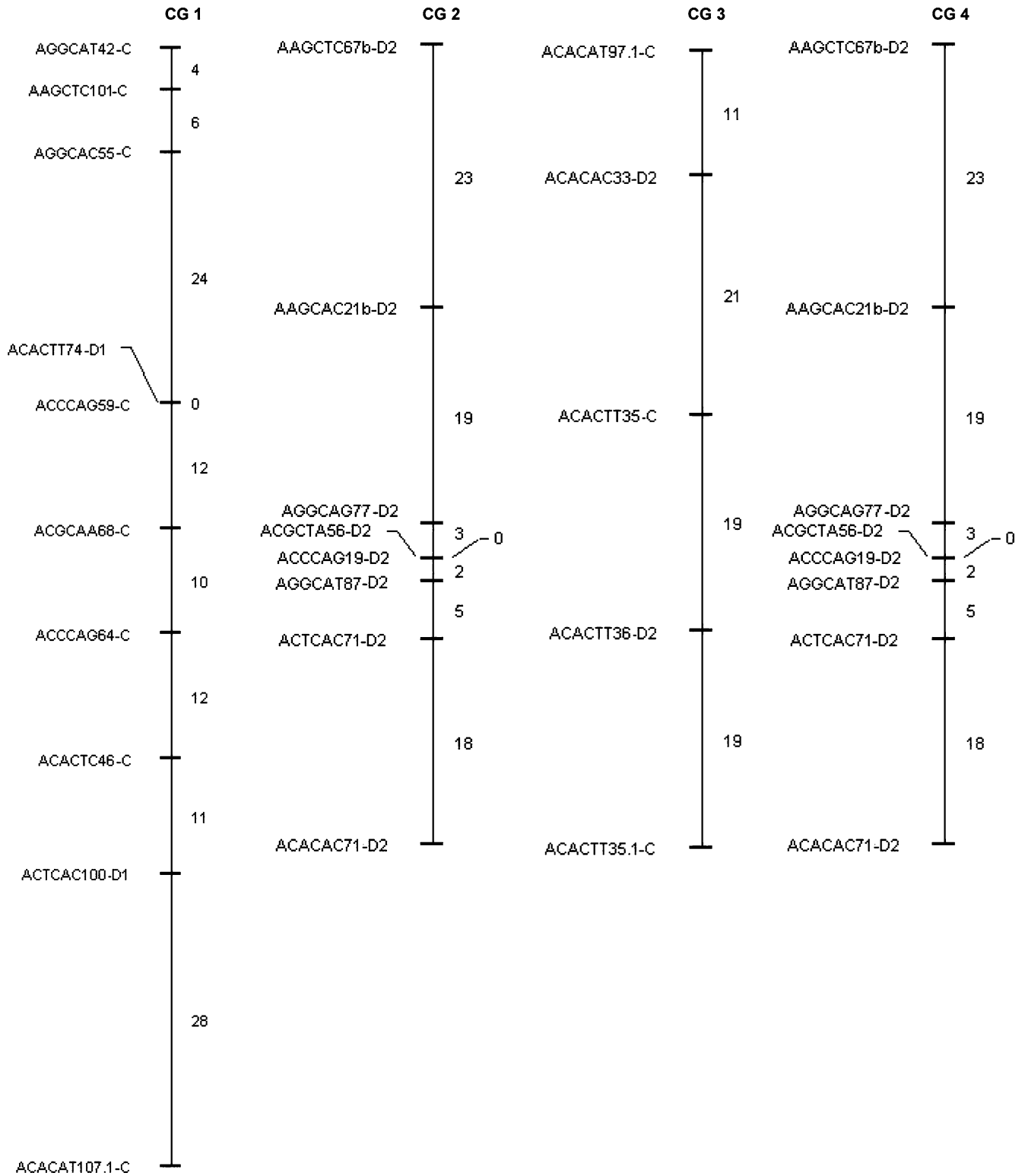
Fig. 1 (Contd.)

analyzing a progeny with 100 individuals. The overall level of single-dose polymorphism achieved (73%) is in agreement with those obtained by various sugarcane segregating populations. Single-dose polymorphisms of 79 and 65% were detected for the selfed-progeny of cultivar R570 with AFLP markers (Hoarau et al. 2001) and resistance gene analogs (Rossi et al. 2003), respectively. The same level of single-dose polymorphism was estimated for an interspecific cross between *S. officinarum* (71%) and *S. robustum* (69%) using AFLP markers

CG 5

AACCTC69b-D2

20

ACACTC68b-D2

22

SG057B-D2

22

SMC236B1-D2

CG 6

AGCCAG19-C

17

AGGCAG27-C
ACACAC69-C
2
8

ACGCAG23-C

21

ACACTT44-C

15

ACACTT61-C

CG 7

ACTCAC28-C

22

AGGCAC74-D2

9

AACCAC21-C

8

ACACAC43-C

11

ACACAT65-C

13

ACCCAG14-C   0
AACCAC29-D2
AACCAC63-C   2

CG 8

ACACTC95-D2

22

ACACTT57-D2

8

AGGCAC61-D2

25

SMC1047-3-D2

CG 9

ACGCAA05-D1

22

ACTCAC37-C
ACACTT41-D1
2

17

ACACTC64-D1

CG 10

AAGCAC77-C

12

AGGCTT37-D2

11

ACTCAC26-D2

17

AGCCAT80-D2

CG 11

ACGCAA41-C

12

ACTCTT26-C

14

ACTCAC72-C

13

ACCCAG12-C

CG 12

ACACAT78-D1
AGGCAC31-D1
2

24

AGCCAT39-D1

20

AGGCAT28-D1

Fig. 1 (Contd.)

CG 13

CIR18-6-C

12

CIR18.7-D1

27

CIR18.5-D1

CG 14

ACTCTT44-C

AACCAC22.-D2 — 5

ACTCAC04-C — 0

9

AACCAT52.1-D2

22

AACCAC07-D2

CG 15

ACGCAG115-C

16

AGCCAG41-C

9

ACT5CAG30-D1

3

AAGCAC73-C

6

ACTCAG30-D1

CG 16

AGGCAT27-C

AGCCAT83-C — 9

AGGCAT15-D2 — 0

AGCCAG13-C — 0

9

AAGCAC51-C

12

ACACTT15-C

CG 17

AGGCAG136-D1

9

AGGCAG74-D1

8

ACCCAC14-C

11

AGCCAG07-D1

CG 18

ACTCTT27-C

14

ACTCAC17.1-C

10

ACACTT96-C

CG 19

ACTCAC58-D2 — 2

AGGCAG07-C — 4

AACCAC69-D2

14

AAGCAC40-C

CG 20

AGGCAC18-D2

8

AGCCAT32-D2

14

ACACTT31-D2

CG 21

ACGCAA62-C

AACCAC24-D1 — 4

AAGCTC34-C — 0

16

AACCAC36-D1

CG 22

AGGCAG41-C

11

ACCCAC39-D2

8

AGGCAG34-C

CG 23

ACGCTA15-D1

ACGCAA24-C — 0

4

AACCAC16-D1

12

AGCCAG39-C

CG 24

AACCTC33-D2 — 3

AACCAT79-C — 4

AGGCAG31-D2 — 7

AACCAT30-C

CG 25

AGGCTT105-D2

5

ACGCAG54-D2

7

ACGCAG04-D2 — 0

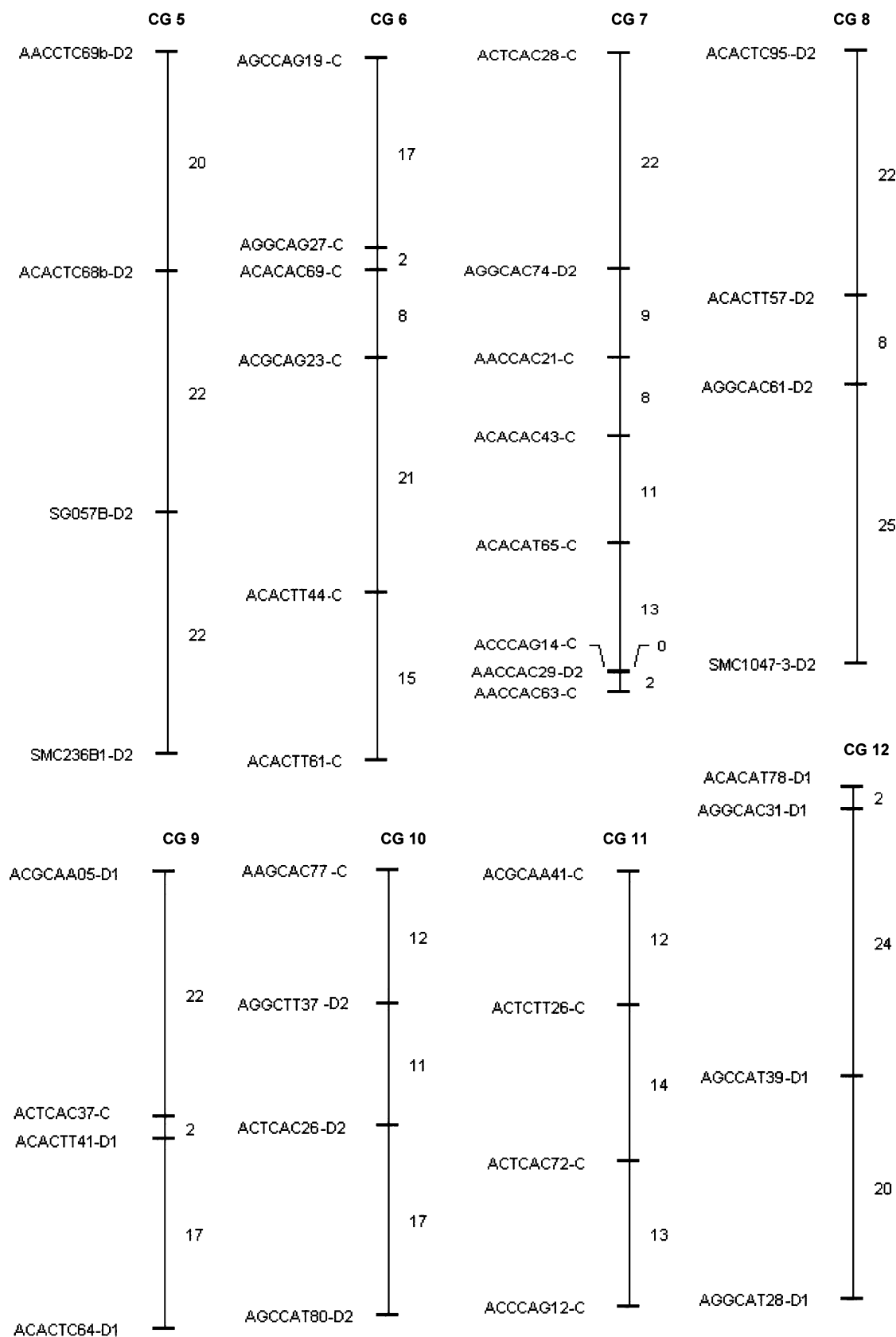ACGCAG03-D2

CG 26

ACTCAC88-C
ACTCTT78-C — 0.5

6

AACCAT67-C

Fig. 1 (Contd.)
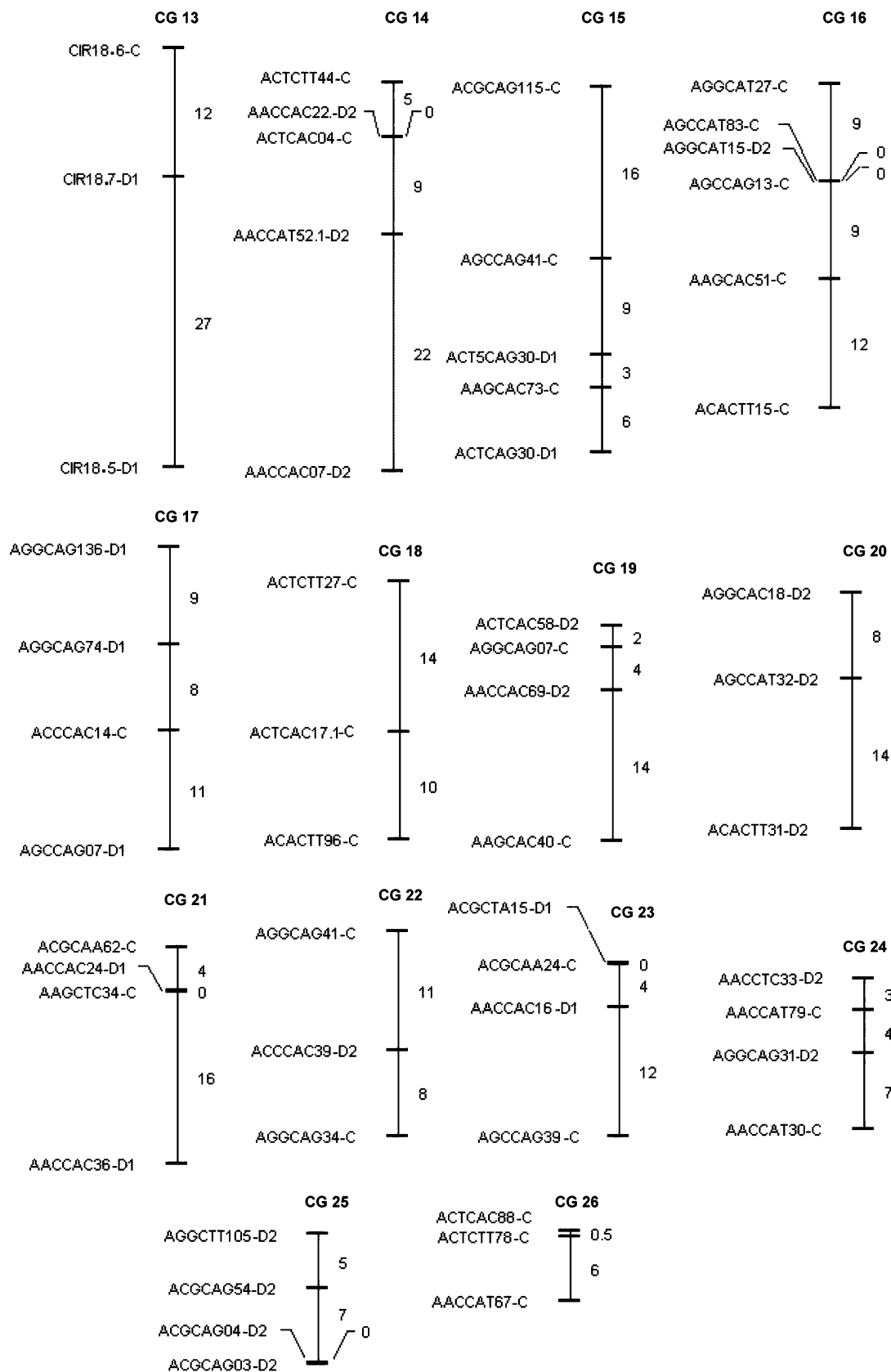
Table 3 Sixty-three co-segregation groups (CGs) with two markers in the integrated genetic map of a sugarcane commercial cross (SP80-180 × SP80-4966). Map distances are given in centi-Morgans (Kosambi)

Markers	Distance
AGGCAC34-D2; SMC765B5-D2	28
SMC119.4-C; SMC119.5-C	28
CIR55.17-D2; CIR55.3-D2	26
SMC2017.1-D1; SMC415.9-D1	26
AGCCAT68-D2; ACGCAA18-D2	25
AGGCTT21-D2; AGCCAT38-D2	24
ACTCAC33-D1; ACGCAG76-D1	24
ACGAA15-C; ACACAC28-C	20
ACACAC90-C; ACACAC68-C	20
AGGCAG17-C; ACACAC56-C	20
AGGCAG118-D2; AGCCAT19-D2	20
AGGCAT13-D2; ACACAT45-D2	19
AGCCAT76-C; ACTCTT42-C	19
AGGCAG45-D1; SG105E-D1	19
AGGCAT26-D1; ACACAC58-D1	17
ACCCAG17-C; AGGCTT90-D2	16
AGGCAT26-D1; ACACAC58-D1	17
ACCCAG17-C; AGGCTT90-D2	16
ACGCAG60-C; ACGCAG59-C	16
ACTCAG16-D2; ACGCAG26-D2	15
ACCCAG31-C; ACACAT24-C	15
ACACAC44-D2; AAGCTC78-D2	14
ACCCAG53-C; AAGCTC22-C	14
ACGCAA39-C; AACCTC52-C	14
ACCCAG09-D1; AAGCTC92-D1	13
ACTCAG33-C; ACGCAA66-C	13
ACCCAG09-D1; AAGCTC92-D1	13
ACCCAG09-D1; AAGCTC92-D1	13
ACTCAC58.1-D2; ACACTT25.1-D2	13
AAGCCAC50-C; AACCAT64.1-C	13
AGCCAG33-C; AGCCAG24-C	12
AAGCAC60-C; ACTCAC52-D1	12
ACGCAG70-C; AACCAC34-C	11
AGGCAG61-D2; ACGCAA23-D2	11
AGGCAC21-C; ACCCAC72-D2	11
AGGCAC38-D1; AACCAC88-D1	10
ACTCAG38-D2; ACACAC48.1-D2	9
ACGCTA33-D2; AACCAC27-D2	9
ACCCAG67-C; ACCCAG50-C	8
AGGCAG12-D1; ACGCTA59-D1	8
ACACTT81-C; AACCAC50-D2	8
AACCTC30-D2; AACCAC83-D2	8
AAGCAC05-C; AAGCAC04-C	7
ACACTC20-C; ACACAT42-C	7
AAGCTC71-C; AAGCAC72-C	7
ACTCTT85-C; AACCAC46-C	7
AGGCAT68-D2; AGGCAG35-D2	7
ACACAT47-C; ACACAC29-C	7
AGGCAG42-C; AGGCAC32-C	6
AGCCAT82-D1; ACTCAC35-D1	4
AGGCTT39-C; ACTCTT73-C	4
AAGCTC96-C; AGGCAG129-D1	3
AACCAT63.1-C; AACCAC08-C	3
ACACAC100-C; AACCAT44.1-D1	2
AGGCAT06-C; ACACAC53-C	1
ACGCAG25-C; AAGCAC70.1-C	0.5
AAGCAC03-D2; AAGCAC02-D2	0
ACACAT54-C; ACCCAC18-D2	0
AGGCAC40-C; AGGCAC01-D2	0
ACACTT23-C; ACTCTT35-D2	0
AGCCAT02-C; AGCCAT01-C	0
ACACTT07-C; AGCCAG37-D2	0
ACGCAG127-C; ACACTT63-D2	0

(Guimarães et al. 1999). Despite the high level of single-dose loci observed among the polymorphic markers, 63% of the monomorphic markers between the mapping parents were double single dose. As reflected by the high percentage (60%) of markers in "$ao \times ao$" cross-type configuration, these double single-dose markers represent alleles shared by the mapping parents. In fact, 'SP80-180' and 'SP80-4966' have some common ancestors. Although heterozygous markers in both parents with a 3:1 segregation ratio are less informative than those partially informative in a testcross configuration (Maliepaard et al. 1997; Wu et al. 2000), the double single-dose markers can serve as a locus bridge to determine homologies of co-segregation groups between the mapping parents i.e., linkage groups as maternal and paternal homologous groups (Grattapaglia and Sederoff 1994).

Integrated map

Basically, the determination of CG depends on the critical LOD score threshold. Sugarcane mapping has been constructed with LOD score values from 3 to 9, and recombination fraction from 0.25 to 0.35 (Al-Janabi et al. 1993; Da Silva et al. 1993; Mudge et al. 1996; Ming et al. 1998; Guimarães et al. 1999; Hoarau et al. 2001). In our integrated linkage map, markers were assigned to CG with a LOD score of 5.0, assuming a recombination fraction of 37.5 cM. These critical values were adopted to avoid incorrect assignment of markers to a CG, considering that the present map will be further used to locate quantitative trait loci.

The map generated using the method proposed by Wu et al. (2002) enabled us to enhance the genome coverage in 1,262.4 cM, by adding 33 CG to the previous 98 CG obtained with JoinMap. The number of CG achieved with the Wu et al. (2002) approach is closer to the $2n = 100$–130 chromosomes, expected for modern sugarcane cultivars (Grivet and Arruda 2001; Hoarau et al. 2001). However, the high number of unlinked markers allied to the small size of most CG and the reduced number of markers (loci) per CG indicates that the map is not saturated. Probably, the small CG likely represents unconnected parts of other groups as a consequence of the higher critical LOD scores adopted. Usually, only single-dose polymorphisms have been selected for mapping (Ming et al. 1998), thus gaps in sugarcane maps are commonly expected. Besides, only linkages in coupling were explored when only single-dose polymorphisms were used for mapping (Al-Janabi et al. 1993; Da Silva et al. 1993). Another possible explanation for the small number of linked markers in our map is that this genetic map is from a cross between two commercial cultivars, which are interspecific hybrids, with a very complex genetic system. It is known that this kind of complex polyploid hybrids has aneuploidy and that there are chromosomes that are not

Table 4 Comparison between the maps generated by different methodologies

Methodology	Number of CG	Number of mapped markers	Number of unmapped markers	Map coverage[a] (cM)	Marker density (cM/marker)
Wu et al. (2002)	131	357	761	2,602.4	7.3
Maliepaard et al. (1997)	98	217	901	1,340.0	6.2

[a]Corresponding to the cumulative length of all CG

paired in the meiosis (Jannoo et al. 1999; Irvine 1999). Making a map in crosses involving this complexity will certainly make it difficult to find linked markers.

The differences in marker order observed between the map developed by JoinMap and the one using the approach proposed by Wu et al. (2002) could be due to intrinsic differences in the algorithms used for ordering markers. JoinMap, a computer program developed to generate genetic linkage maps in experimental populations of diploid species, uses mean recombination frequencies and combined LOD scores for map calculations (Stam 1993). The methodology developed by Wu et al. (2002) has an objective criterion to distinguish the linkage phases between markers based on the posterior probability of each linkage phase. Thus, it allows the simultaneous estimation of the recombination fraction and the linkage phase, solving several statistic drawbacks pointed out in JoinMap and discussed by Maliepaard et al. (1997). Another advantage of the Wu et al. (2002) approach is that the method allows multipoint analysis for the recombination fraction between linked markers based on the likelihood that in turn significantly improves the estimates resulting in more reliable maps (Lander and Green 1987). In our case, such an approach allowed us to precisely construct the co-segregation groups with the higher number of markers, which was not achieved using JoinMap. Therefore, the theoretical advantages of the Wu et al. (2002) approach were confirmed in practice when our data set was analyzed.

Linkage between D_1 markers (SP80-180 origin) and D_2 (SP80-4966 origin) was not observed in both mapping approaches (JoinMap or simultaneous maximum-likelihood estimation of recombination and linkage phases) since these markers, between them, are not informative (Maliepaard et al. 1997; Wu et al. 2002). One exception was marker SMC36A16-D_1, which was linked with D_2 markers in HG-I (I-1). The CGs represent the meiosis, which occurred in the parents. It is not expected that markers exclusively derived from the female parent (D_1 type) and from the male parent (D_2 type) to be linked in the same CG, unless markers common to both parents ("C" type) appear linked with the other types. Despite the large number of "C" type markers (symmetrical marker cross type) detected, they were not sufficient to establish linkage between many cross type D_1 and D_2 markers.

The linkage map presented here is the first one to our knowledge to apply the method described by Wu et al. (2002) to construct a single map of a full-sib family de-

rived from the cross between two pre-commercial sugarcane cultivars. Expressed sequence tags (EST) derived markers obtained from the Sugarcane Expressed Sequence Tag (SUCEST) Project (Vettore et al. 2001) are being added to the map constructed with anonymous markers (Pinto et al. 2004). Since ESTs represent expressed genes, the EST derived markers will allow us to construct a useful sugarcane map that will facilitate QTL mapping via a candidate gene approach.

Homology groups

Markers derived from the same SSR locus or RFLP probe can identify co-segregation groups corresponding to homologous chromosomes (Grivet and Arruda 2001). The number of homology groups is associated with the basic number of chromosomes of the genus *Saccharum* (Irvine 1999). In fact, sugarcane homologous groups identified on the basis of common RFLP probes have ranged between 8 (Da Silva et al. 1993) and 10 (Grivet et al. 1996). Rossi et al. (2003) assembled 66 CG into seven HGs based on SSR and RGA markers. In our case, 131 CG were assigned to 12 HGs, slightly above the expected basic number for the genus. Most HGs were formed with small CGs (with only two markers) contributing to assemble CGs in different HGs. Markers derived from a common SSR locus or RFLP probe linked in the same CG probably correspond to duplicated loci. Of the 131 CG formed 14 (11%) could contain duplicated loci. Duplicated small genome regions have been reported in the majority of sugarcane maps developed with co-dominant markers (Da Silva et al. 1993, 1995; Dufour et al. 1997; Ming et al. 1998). These regions probably occur due to genome heterogeneity between the ancestral species (*S. officinarum* and *S. spontaneum*), which has distinct basic chromosome numbers and may represent genome structural rearrangements derived from translocations or associated with transposable element activity observed in polyploids (D'Hont et al. 1998, Jannoo et al. 2004). Moreover, the unsystematic meiotic behavior in sugarcane led to pairing affinities between chromosomes determined by its individual features, which are related to ancestral origin (Jannoo et al. 2004).

Breeding applications

Commercial sugarcane clones obtained through breeding are selected from a population derived from artificial

crosses between two (bi-parental crosses) or several elite cultivars (poly-crosses). During selection, individuals are vegetatively propagated and field evaluated through several stages before being released as commercial cultivars. Selection for superior clones is, therefore, a long and expensive process, taking up to 12 years (Calija et al. 2001) that can benefit from the application of molecular techniques. In this aspect, linkage maps of progenies derived from elite materials segregating for important sugarcane traits such as disease resistance and sugar content can be helpful to implement MAS (Marker Assisted Selection) in sugarcane breeding programs. The genetic map of the R570 cultivar (Grivet et al. 1996) allowed the identification of a RFLP marker (CDSR29 probe) linked to a putative gene for rust resistance (*Puccinia melanocephala*) that segregated at a 3:1 ratio (resistance:susceptibility) in the progeny (Daugrois et al. 1996). Forty putative QTAs (Quantitative Trait Alleles) were detected for yield components such as plant height, stalk number, stalk diameter and brix in the mapping population derived from R570 (Hoarau et al. 2002). QTA validation using different cultivars or species will increase the certainty of their existence and hence, the markers linked to them will be useful in other germplasms. However, polyploidy is still a challenge for the application of molecular markers in sugarcane, more than in any other crop. Specifically, biometrical methods are still necessary to extract information in QTA experiments (Grivet and Arruda 2001).

References

Al-Janabi SM, Honeycutt RJ, McClelland M, Sobral BWS (1993) A genetic linkage map of *Saccharum spontaneum* L, 'SES 208'. Genetics 134:1249–1260

Barreneche T, Bodenes C, Lexer C, Trotin JF, Fluch S, Streiff R, Plomion C, Roussel G, Steinkellner H, Burg K, Favre JM, Glössl J, Kremer A (1998) A genetic linkage map of *Quercus robur* L. (pendunculate oak) based on RAPD, SCAR, microsatellite, minisatellite, isozyme and 5S rDNA markers. Theor Appl Genet 97:1090–1103

Buetow KH, Chakravarti A (1987) Multipoint gene mapping using seriation. I. General Methods. Am J Hum Genet 41:180–188

Burnquist WL (1991) Development and application of FRLP technology in sugarcane (Saccharum sp) breeding. PhD Dissertation, Cornell University, Ithaca NY, 154 p

Calija V, Higgins AJ, Jackson PA, Bielig LM, Coomans D (2001) An operations research approach to the problem of the sugarcane selection. Ann Oper Res 108:123–142

Carlier JD, Reis A, Duval MF, Coppens D'Eeckenbrugge G, Leitão M (2004) Genetic maps of RAPD, AFLP and ISSR markers in *Ananas bracteatus* and A. comosus using the pseudotestcross strategy. Plant Breed 123:186–192

Cordeiro GM, Taylor GO, Henry RJ (2000) Characterization of microsatellite markers from sugarcane (*Saccharum* sp.) a highly polyploid species. Plant Sci 155:161–168

Creste S, Tulmann Neto A, Figueira A (2001) Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gel by silver staining. Plant Mol Biol Rep 19: 299–306

Da Silva JAG, Sorrells ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36:782–791

Da Silva JAG, Honeycutt RJ, Burnquist WL, Al-Janabi SM, Sorrels ME, Tanksley SD, Sobral BWS (1995) *Saccharum spontaneum* L. 'SES208' genetic linkage map containing RFLP and PCR-based markers. Mol Breed 1:165–169

Daugrois JH, Grivet L, Roques D, Hoarau JY, Lombard H, Glaszmann JC, D'Hont A (1996) A putative major gene for rust resistance linked with RFLP marker in sugarcane cultivar R570. Theor Appl Gen 92:1059–1064

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via KM algorithm. J R Stat Soc Ser B 39:1–38

D'Hont A, Ison D, Alix K, Roux C, Glaszmann JC (1998) Determination of the basic chromosome numbers in the genus *Saccharum* by physical mapping of RNA genes. Genome 4:221–225

D'Hont A, Lu YH, De Léon DG, Grivet L, Feldmann P, Lanaud C, Glaszmann JC (1994) A molecular approach to unravelling the genetics of sugarcane, a complex polyploid of the Andropogoneae tribe. Genome 37:222–230

Doerge RW (1996) Constructing genetic maps by rapid chain delineation. J Agric Genomics 2:6

Dufour P, Deu M, Grivet L, D'Hont A, Paulet F, Bouet A, Anaud C, Glaszmann JC, Hamon P (1997) Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploidy. Theor Appl Genet 94:409–418

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 127:1121–1137

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann C (1996) RFLP Mapping in cultivated sugarcane (*Saccharum* spp.): Genome organization in a highly polyploid and aneuploid interspecific hybrid. Genetics 142:987–1000

Grivet L, Arruda P (2001) Sugarcane genomics: depicting the complex genome of an important tropical crop. Curr Opin Plant Biol 5:122–127

Guimarães CT, Honeycutt RJ, Sills GR, Sobral BWS (1999) Genetic maps of *Saccharum officinarum* L. and *Saccharum robustum* Brandes and Jew. Ex. Grassl. Genet Mol Biol 22:125–132

Hoarau JY, Grivet L, Offmann B, D'Hont A, Risterucci AM, Roques D, Glaszmann JC, Grivet L (2001) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp.) I. Genome mapping with AFLP markers. Theor Appl Genet 103:84–97

Hoarau JY, Grivet L, Offmann B, Raboin LM, Diorflar JP, Payet J, Hellmann M, D'Hont A, Glaszmann JC (2002) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp) II Detection of QTLs for yield components. Theor Appl Genet 105:1027–1037

Hoisington D, Khairallah M, González-De-León D (1994) Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, Mexico, DF

Irvine JE (1999) Saccharum species as horticultural classes. Theor Appl Genet 98:186–194

Jannoo N, Grivet L, D'Hont A, Glaszmann JC (2004) Differential chromosome pairing affinities at meiosis in polyploid sugarcane revealed by molecular markers. Heredity 93:460–467

Jannoo N, Grivet L, Seguin M, Paulet F, Domaingue R, Rao PS, Dookun A, D'Hont A, Glazmann JC (1999) Molecular investigation of the genetic base of sugarcane cultivars. Theor Appl Genet 99:171–184

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci 84:2363–2367

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lima MLA, Garcia AAF, Oliveira KM, Matsuoka S, Arizono H, Souza CL Jr, Souza AP (2002) Analysis of genetic similarity detected by AFLP and coefficient of parentage among genotypes of sugar cane (Saccharum spp.) Theor Appl Genet 104:30–38

Liu BH (1998) Statistical Genomics. CRC Press NY, 611 pp

Maliepaard C, Jansen J, Van Ooijen JW (1997) Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genet Res 70:237–250

Maliepaard C, Alston FH, Van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, Van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, Den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink Van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73

Ming R, Liu SC, Lin YR, Da Silva J, Wilson W, Braga D, Van Deynze A, Wenslaff TF, Wu KK, Moore PH, Burnquist W, Sorrells ME, Irvine JE, Paterson AH (1998) Detailed alignment of Saccharum and Sorghum chromosomes: Comparative organization of closely related diploid and polyploid genomes. Genetics 150:1663–1682

Mudge J, Andersen WR, Kehrer R, Fairbanks DJ (1996) A RAPD genetic map of Saccharum officinarum. Crop Sci 36:1362–1366

Pinto LR, Oliveira KM, Ulian EC, Garcia AAF, Souza AP (2004) Survey in the sugarcane expressed sequence tag data base (SUCEST) for simple sequence repeats. Genome 47:795–804

Porceddu A, Albertini E, Barcaccia G, Falistorco E, Falcinelli M (2002) Linkage mapping in apomictic and sexual kentucky blue grass (Poa pratensis L) genotypes using a two way pseudo-testcross strategy based on AFLP and SAMPL markers. Theor Appl Genet 104:273–280

R Development Core Team, R (2004) A language and environment for statistical computing, Foundation for Statistical Computing.Vienna, Austria 3–900051-07-0 (http://www.R-project.org)

Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H, Van Sluys MA, D'Hont AD (2003) Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. Mol Gen Genomics 269:406–419

Shepherd M, Cross M, Dieters MJ, Henry R (2003) Genetic maps for Pinus elliottii var hondurensis using AFLP and microsatellite markers. Theor Appl Genet 106:1409–1419

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Van Ooijen JW, Voorrips RE (2001) JoinMap Version 3.0 Software for the calculation of genetics linkage maps. Plant Research International, Wageningen, The Netherlands

Vettore AL, Da Silva FR, Kemper EL, Arruda P (2001) The libraries that made SUCEST. Genet Mol Biol 24:1–7

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 83:294–300

Wu RL, Han YF, Hu JJ, Fang JJ, Li L, Li ML, Zeng ZB (2000) An integrated genetic map of Populus deltoides based on amplified fragment length polymorphisms. Theor Appl Genet 100:1249–1256

Wu R, Ma CX, Painter I, Zeng ZB (2002) Simultaneous maximum likelihood estimation of linkage and linkage phases in outcrossing species. Theor Popul Biol 61:349–363