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An assessment of the genetic diversity within a collection of *Saccharum spontaneum* L. with RAPD-PCR★

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Abstract

A local collection of 33 *Saccharum spontaneum* L. clones and two sugarcane cultivars (LCP 82-89 and LCP 85-384) were assessed for genetic variability using random amplified polymorphic DNA (RAPD)-PCR. A total of 157 polymorphic RAPD-PCR bands were scored with 17 primers. The number of RAPD-PCR products per primer ranged from four to 16. The data were analyzed with two multivariate analysis software programs, NTSYSpc and DNAMAN®. Although these two programs yielded similar results, a bootstrapped phylogenetic tree could only be generated with the DNAMAN® software. A substantial degree of genetic diversity was found within the local *S. spontaneum* collection. Pairwise genetic homology coefficients ranged from 65% (SES, 196/Tainan $2n = 96$) to 88.5% (IND 81-80/IND 81-144). LCP 82-89 and LCP 85-384 shared a greater similarity (82%) than either was to any clone of *S. spontaneum* (ranging from 60.5 to 75.2%). The 33 *S. spontaneum* clones were assigned to eight groups independent of their geographic origin or morphology, while the two sugarcane cultivars were assigned to the ninth group. All but two pairs of *S. spontaneum* clones could be distinguished by a single RAPD primer OPBB-02. The use of a second primer, either OPBE-04 or Primer 262, separated all *S. spontaneum* clones. One amplification product from the RAPD primer OPA-11, OPA-11-336, proved to be cultivar-specific and has been adopted for use in our breeding program. Information from this study would help conserve the genetic diversity of *S. spontaneum*.

Introduction

Sugarcane cultivars (*Saccharum* hybrids) are believed to be aneupolyploid hybrids of *S. officinarum* L. (Linnaeus 1753; Grassl 1969), *S. barberi* Jeswiet (Brandes 1958), *S. sinense* Roxb. (Brandes 1958; Roxburgh 1819), *S. robustum* Brandes and Jeswiet ex Grassl (Grassl 1946), and *S. spontaneum* L. (Linnaeus 1771). Recently, Ming et al. (1998) hypothesized that only two species, *S. robustum* and *S. spontaneum*, were the progenitors of modern sugarcane, that *S. officinarum* may be derived from *S. robustum*, and that *S. barberi* and *S. sinense* were cultivated forms of interspecific hybrids between *S. spontaneum* and *S. officinarum*. Almost all sugarcane cultivars grown in the world

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Table 1. Saccharum spontaneum clones from the local collection at the USDA-ARS Sugarcane Research Unit at Houma, Louisiana.

Accession	Origin[a]	Accession	Origin[a]
Coimbatore	India	PCAV 84-12	Philippines
Djatiroto	USA	PIN 84-1	Philippines
Gehra Bon	India	PPGN 84-7	Philippines
Holes	India	SES 6	India
IN 84-21	Indonesia	SES 84/58	India
IN 84-58	Indonesia	SES 147B	India
IND 81-080	India	SES 184B	India
IND 81-142	India	SES 189	India
IND 81-144	India	SES 196	India
IND 81-161	India	SES 205A	India
IND 81-166	India	SES 231	India
IND 82-257	India	SES 234	Malaysia
IND 82-311	India	SES 323	India
Mol 1032	Australia	SH 249	India
S 66-084	Taiwan	Tainan $2n = 96$	Taiwan
S 66-121	Taiwan	US 56-15-8	USA
PCANOR 84-2	Philippines		

[a]USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN) <http://www.ars-grin.gov/npgs/>.

today are derived from a few common ancestral clones and therefore share a limited genetic base (Arceneaux 1967; Tew 1987, 2003). Nonetheless, elite cultivars could be grouped by microsatellite fingerprints (Pan et al. 2002) or by the presence or absence of *S. spontaneum* alleles using RFLP analysis (Jannoo et al. 1999). A common objective for many breeding programs in the world is to expand the genetic base of sugarcane by introducing agriculturally desirable traits from related wild species, particularly *S. spontaneum* (Legendre and Breaux 1983; Burner and Legendre 1993). *Saccharum spontaneum* is the most widely used species in the breeding program at the USDA-ARS, Sugarcane Research Unit because of its stubble vigor, longevity, cold tolerance, and disease and insect resistance (Dunckelman and Legendre 1982).

Various ecotypes of *S. spontaneum* can be found in the wild from Africa to Asia and across the Pacific islands (Artschwager and Brandes 1958). Chromosome numbers of this species range from $2n = 40$ to 128 (for a review, see Sreenivasan et al. 1987). Extensive variations in physiological and morphological traits were also observed by many sugarcane breeders in their *S. spontaneum* collections (Chu et al. 1962; Dunckelman and Breaux 1969; Kandasami et al. 1983; Nagatomi and Ohshiro 1983; Rao and Vijayalakshmi 1963; Tai et al. 1995). In the last decade, RAPD-PCR markers were used to assess the genetic diversity in elite and exotic germplasm (Burner et al. 1997; Harvey and Botha 1996; Harvey et al. 1994; Pan et al. 1997), confirm cultivar pedigree (Huckett and Botha 1995), construct genetic maps (Mudge et al. 1996; Sobral and Honeycutt 1993), define linkages with quantitative traits (Msomi and Botha 1994; Sills et al. 1995), and assess the extent of self-pollination in sugarcane crossing (McIntyre and Jackson 1995). Very few if any of the studies mentioned above involved *S. spontaneum*. Thus, there is a gap in the knowledge base concerning the application of RAPD technology to this important germplasm. The objective of this study was to assess the genetic variability within a local collection of *S. spontaneum* with RAPD-PCR fingerprints. RAPD-PCR fingerprints were analyzed using both NTSYSpc (Exeter Software, Setauket, NY) and DNAMAN® software. The DNAMAN® software allowed the production of a phylogenetic tree with bootstrapping (confidence) values.

Materials and method

Thirty-three clones of *S. spontaneum* (Table 1), and two sugarcane cultivars, LCP 82-89 (Martin et al. 1992) and LCP 85-384 (Milligan et al. 1994), were included in the study. Twelve plants were

Table. 2 Identity, sequence, and number of polymorphic bands for RAPD primers tested on *Saccharum spontaneum* clones.

Primer	Sequence (5′–3′)	# of polymorphic bands observed
KO2T	CCGAATTCGCC[a]	10
OPA-l0	GTGATCGCAG	11
OPA-l1	CAATCGCCGT	7
OPA-17	GACCGCTTGT	11
OPAD-01	CAAAGGGCGG	16
OPAD-13	GGTTCCTCTG	4
OPAH-09	AGAACCGAGG	8
OPAX-20	ACACTCGGCA	6
OPBB-02	CCCCCGTTAG	14
OPBB-18	CAACCGGTCT	6
OPBE-04	CCCAAGCGAA	9
OPBE-07	CCGTCCTATG	10
OPBE-09	CCCGCTTTCC	7
OPC-04	CCGCATCTAC	8
OPN-19	GTCCGTACTG	9
OPY-17	GACGTGGTGA	7
262	CGCCCCCAGT[b]	14

[a]Tao et al. (1993).
[b]Fritsch et al. (1993).

established from the selfed seeds of each *S. spontaneum* clone and of LCP 82-89. These plants were maintained in a greenhouse at the Sugarcane Research Unit, Houma, Louisiana. Another 12 plants of LCP 85-384 were propagated from vegetative buds. The leaf whorl from individual plants was used for DNA extraction. Total DNA was extracted from about 200 mg of fresh leaf tissue according to Pan et al. (2000). Equal volumes of total DNA from the 12 plants within each clone or variety were pooled, and 1 μL of the pooled DNA sample was diluted in 49 μL sterile water.

Sixteen 10-mer and one 11-mer RAPD primers (Table 2) were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) or Operon Technologies, Inc. (Alameda, CA) for RAPD-PCR amplification. Primers were chosen randomly from the RAPD primer collection, except for KO2T and 262 that, previously, were known to generate polymorphic products in various plant taxa (Fritsch et al. 1993; Tao et al. 1993) including sugarcane (Burner et al. 1997). Each primer was diluted in sterile water to a concentration of 100 pM/μL and stored in aliquots at $-20\,^{\circ}$C. RAPD reactions and gel electrophoresis were conducted according to Pan et al. (1997). The molecular weights of RAPD-PCR bands were determined using the DNA size markers (Cat. #P9577, Sigma Chemical Co., St. Louis, MO; or Cat. #10380-020, Gibco-BRL, Gaithersburg, MD) with GelExpert™ software on a Gel Documentation Station (NucleoTech Corp., Hayward, CA). The RAPD analysis was repeated for primers OPBB-02, OPBE-04, and Primer 262. The amplified DNA bands from these repeated runs were used to examine the reproducibility of RAPD-PCR and to determine the minimum number of additional primers required to distinguish among the 33 *S. spontaneum* clones and the two sugarcane cultivars.

The distribution of each of the 157 RAPD-PCR bands in each *S. spontaneum* clone or sugarcane cultivar was recorded either as present [a value of 1 for NTSYSpc (Exeter Software, Setauket, NY) or the letter A for DNAMAN® (Lynnon Biosoft, Vaudreuil, Quebec, Canada)] or absent (a value of 0 for NTSYSpc or the letter C for DNAMAN®). As a result, each sample was represented by either a random array of 157 1 s or 0 s for NTSYSpc or an arbitrary 157-base long DNA sequence of As and Cs for DNAMAN®. Pairwise genetic distance or homology coefficients were calculated using the observed divergence method by both software programs. Multiple sequence alignment was conducted with DNAMAN® using an optimal alignment method (Feng and Doolittle 1987; Thompson et al. 1994) and a multiple sequence editor (MASED). MASED produces homology and phylogenetic trees in graphic windows. The phylogenetic tree was constructed with the distance matrix, which is equal to 1 minus the homology value, based on the neighbor-joining algorithm of Saitou and Nei (1987).

Results

RAPD reactions were all successful except for one reaction in which the clone IND 82-311 failed to amplify with primer OPBE-07. The number of RAPD-PCR bands per primer ranged from 4 to 16. In total, 157 RAPD-PCR polymorphic bands were scored with the 17 primers (Table 2). In general, there was a high level of reproducibility between repeated RAPD-PCR runs. For primer 262, identical banding patterns were observed from repeated runs on all samples. The same was true for primer OPBE-04 except on clones SES 196 and

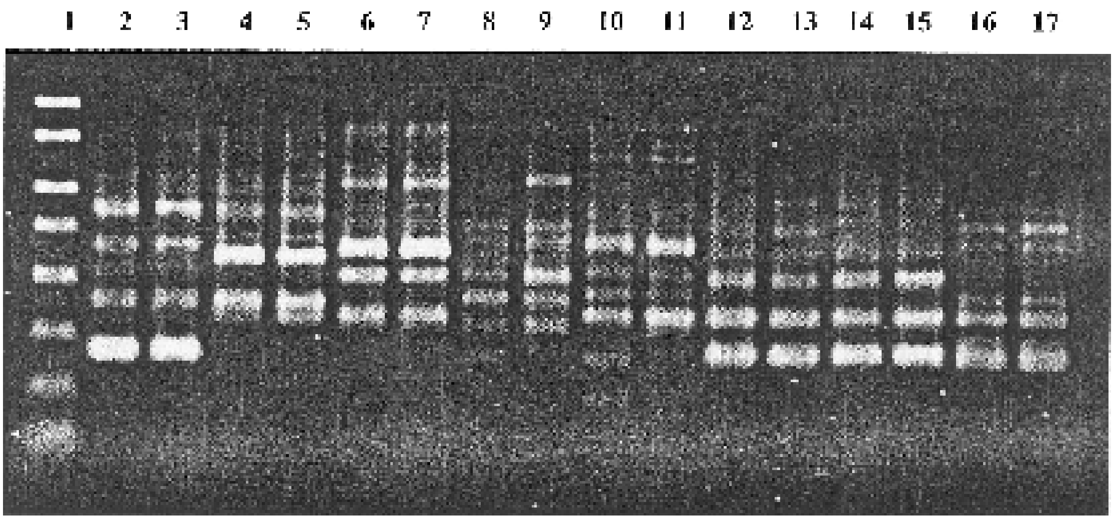

Figure 1. Ethidium bromide-stained 1.5% agarose gel of RAPD-PCR products from duplicate runs of OPBB-02-primed amplifications with samples: Lanes 2 and 3: IND 81-166; 4 and 5: SES 189; 6 and 7: IND 81-142; 8 and 9: SES 231; 10 and 11: SH 249; 12 and 13: IND 81-80; 14 and 15: IND 81-144; and 16 and 17: LCP 85-384, respectively. Lane 1 contained DNA size markers (from top down) at 2000, 1500, 1000, 750, 500, 300, 150 and 50 bp, respectively (Cat. No. P9577, Sigma Chemical Co., St. Louis, MO).

IND 81-144, where non-reproducible bands were observed. For primer OPBB-02, non-reproducible bands were observed from clones PCANOR 84-2, SES 231, and SH 249. Primer OPBB-02 was the most polymorphic (Figure 1). With this primer, all but two pairs of *S. spontaneum* clones, IND 81-80/ IND 81-144 and Mol 1032/PPGN 84-7, produced unique banding patterns. In addition, 22 clones produced unique banding patterns with primer 262 and 15 clones produced unique banding patterns with primer OPBE-04.

Although similar results were obtained with NTSYSpc and DNAMAN®, it was only possible to generate a bootstrapped phylogenetic tree with DNAMAN®. Only the data from DNAMAN® are presented here. A pairwise homology matrix among the 33 *S. spontaneum* accessions and two sugarcane cultivars is presented in Table 3. Within the *S. spontaneum* collection, the values ranged from relatively similar (clones IND 81-80 and IND 81-144 with a coefficient of 88.5%) to relatively dissimilar (clones SES 196 and Tainan $2n = 96$ with a coefficient of 65%). The two sugarcane cultivars, LCP 82-89 and LCP 85-384, showed greater similarity to each other (coefficient 82.2%) than either one was to any *S. spontaneum* clone. LCP 82-89 was the most distant to SES 234 and IND 81-166 (coefficient 61.8%), while LCP 85-384 was most dissimilar to IND 81-166 (coefficient 60.5%).

Based on the pairwise genetic distance coefficients, a phylogenetic tree was built by the DNAMAN® program that showed three features: weight of each sequence, branch length (a reference standard of 0.05 was given), and bootstrap values. In this tree, the 33 *S. spontaneum* clones and two sugarcane cultivars were clustered into nine distinct groups (Figure 2). Group I included clones SES84/58 and Tainan $2n = 96$. Group II included clones PCAV84-12, PIN84-1, S66-121, and S66-84. Group III included US56-15-8 and Holes. Group IV included GEHRA BON, IN84-58, IN84-21, PPGN84-7, COIMBATORE, MOL1032, and SES147B. Group V included PCANOR84-2 and SES234. Group VI included IND82-257, IND82-311, SES184B, SES6, SES196, and SH249. Group VII included Djatiroto, SES205A, IND81-161, IND81-166, and SES189. Group VIII included SES233, IND81-144, IND81-80, and IND81-142. Group IX comprised the two sugarcane cultivars. Clone SES231 has its own characteristics. Although it was more closely related to Groups

Table 3. Pairwise homology matrix (%) for 33 *Saccharum spontaneum* accessions and two elite sugarcane accessions.

Accession	Code	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
IND 81-144	1	100																		
IND 81-80	2	88.5	100																	
IND 81-142	3	76.4	79.0	100																
SES 323	4	75.2	75.2	79.6	100															
IND 82-257	5	79.0	76.4	78.3	78.3	100														
IND 82-311	6	72.0	74.5	72.6	77.7	100	100													
SES 6	7	76.4	73.9	75.8	78.3	4.7	79.0	100												
SES 184B	8	73.9	75.2	74.5	75.8	78.3	75.2	80.9	100											
Djatiroto	9	77.1	74.5	76.4	76.4	82.8	75.8	81.5	77.7	100										
SES 205A	10	73.2	68.2	73.9	77.7	79.0	72.0	75.2	70.1	83.4	100									
SES 189	11	76.4	72.6	75.8	78.3	80.9	77.7	80.9	75.8	84.1	80.3	100								
IND 81-161	12	78.3	78.3	77.7	79.0	79.0	78.3	76.4	75.2	83.4	72.0	85.4	100							
IND 81-166	13	74.5	70.7	75.2	75.2	80.3	77.1	77.7	73.9	82.2	74.5	86.6	84.7	100						
PPGN 84-7	14	70.7	69.4	75.2	77.7	79.0	79.6	81.5	76.4	75.8	79.6	79.0	75.8	79.6	100					
Coimbatore	15	67.5	68.8	70.7	79.6	78.3	77.7	79.6	78.3	77.7	79.0	77.1	73.9	76.4	87.9	100				
MOL 1032	16	71.3	72.6	75.8	78.3	78.3	80.3	80.9	72.0	76.4	77.7	75.8	76.4	71.3	85.4	82.2	100			
Gehra Bon	17	66.2	67.5	74.5	74.5	78.3	76.4	78.3	74.5	73.9	72.6	77.1	71.3	73.9	80.3	79.6	80.9	100		
IN 84-21	18	72.0	66.9	73.9	77.7	76.4	74.5	76.4	71.3	73.2	77.1	79.0	73.2	73.2	83.4	79.0	82.8	82.8	100	
IN 84-58	19	67.5	68.8	72.0	77.1	75.8	79.0	77.1	69.4	70.1	71.3	75.8	73.9	75.2	84.1	77.1	79.6	82.2	81.5	100
SES 147B	20	70.1	68.8	70.7	77.1	74.5	72.6	80.9	70.7	72.6	73.9	73.2	68.8	68.8	79.0	80.9	77.1	78.3	79.0	77.1
PCANOR 84-2	21	72.6	70.1	74.5	74.5	79.6	76.4	79.6	73.2	75.2	79.0	74.5	68.8	73.9	84.1	79.6	77.1	80.9	80.3	77.1
SES 234	22	73.2	73.2	75.2	72.6	81.5	72.0	77.7	70.1	79.6	77.1	77.7	70.7	74.5	77.1	76.4	75.2	80.3	77.1	73.9
S 86-44	23	70.7	72.0	72.6	81.5	76.4	79.6	76.4	73.9	72.0	72.0	75.2	72.0	77.1	82.2	79.0	76.4	80.3	79.6	81.5
S 66-121	24	67.5	70.1	70.7	74.5	73.2	77.7	73.2	66.9	67.5	70.1	70.7	68.8	73.9	79.0	74.5	75.8	75.8	76.4	78.3
PIN 84-1	25	75.2	70.1	68.2	75.8	74.5	76.4	73.2	74.5	73.9	73.9	74.5	75.2	75.2	82.8	77.1	73.2	74.5	77.7	79.6
PCAV 84-12	26	72.0	68.2	71.3	75.2	73.9	77.1	72.6	73.9	69.4	69.4	71.3	70.7	73.2	80.9	73.9	70.1	73.9	78.3	76.4
SH 249	27	78.3	75.8	73.9	73.9	77.7	75.8	80.3	75.2	74.5	72.0	72.6	75.8	68.2	74.5	73.9	73.9	72.6	75.8	75.2
SES 196	28	73.9	73.9	66.9	75.8	79.6	77.7	78.3	73.2	73.9	68.8	73.2	72.6	72.6	71.3	70.7	73.2	70.7	70.1	75.8
SES 231	29	76.4	76.4	77.1	75.8	75.8	76.4	77.1	77.1	79.0	76.4	77.1	77.7	71.3	75.2	74.5	75.8	77.1	75.2	72.0
SES 84-58	30	75.8	72.0	73.9	75.2	77.7	72.0	72.6	73.9	82.2	80.9	80.3	74.5	78.3	75.8	76.4	75.2	71.3	75.8	70.1
Tainan $2n = 96$	31	69.4	68.2	73.9	79.0	75.2	72.0	70.1	71.3	74.5	78.3	76.4	74.5	73.2	79.6	76.4	76.4	73.9	79.6	73.9
US 56-15-8	32	70.1	72.6	74.5	75.8	79.6	75.2	77.1	68.2	75.2	71.3	73.2	71.3	68.8	76.4	75.8	79.6	78.3	77.7	70.7
Holes	33	70.7	68.2	75.2	75.2	76.4	72.0	77.7	70.1	73.2	72.0	75.2	72.0	70.7	77.1	72.6	72.6	73.9	77.1	75.2
LCP 85-384	34	64.3	66.9	65.0	67.5	65.0	69.4	66.2	66.2	65.6	66.9	65.0	64.3	60.5	66.9	70.1	70.1	67.5	68.2	63.7
LCP 82-89	35	66.9	66.9	63.7	68.8	66.2	70.7	66.2	65.0	64.3	63.1	62.4	64.3	61.8	66.9	67.5	68.8	65.0	69.4	65.2

Accession	Code	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
IND 81-144	1															
IND 81-80	2															
IND 81-142	3															
SES 323	4															
IND 82-257	5															
IND 82-311	6															
SES 6	7															
SES 184B	8															
Djatiroto	9															
SES 205A	10															
SES 189	11															
IND 81-161	12															
IND 81-166	13															
PPGN 84-7	14															
Coimbatore	15															
MOL 1032	16															
Gehra Bon	17															
IN 84-21	18															
IN 84-58	19															
SES 147B	20	100														
PCANOR 84-2	21	75.8	100													
SES 234	22	76.4	82.8	100												
S 66-84	23	76.4	80.3	78.3	100											
S 66-121	24	74.5	80.9	73.9	85.4	100										
PIN 84-1	25	75.8	78.3	71.3	81.5	75.8	100									
PCAV 84-12	26	70.1	77.7	68.2	83.4	79.0	87.9	100								
SH 249	27	76.4	75.2	74.5	74.5	70.1	73.9	73.2	100							
SES 196	28	73.2	73.2	70.1	71.3	68.2	72.0	68.8	77.7	100						
SES 231	29	73.2	75.8	80.3	77.7	72.0	72.0	71.3	77.7	73.2	100					
SES 84/58	30	68.8	75.2	77.1	74.5	70.1	71.3	68.2	68.2	66.2	75.2	100				
Tainan $2n = 96$	31	71.3	73.9	74.5	80.9	73.9	79.0	77.1	70.7	65.0	71.3	84.7	100			
US 56-15-8	32	74.5	75.8	75.2	76.4	74.5	72.0	73.9	71.3	70.7	72.0	70.1	75.2	100		
Holes	33	72.6	75.2	72.0	74.5	70.1	68.8	72.0	75.8	73.9	75.2	70.7	74.5	76.4	100	
LCP 85-384	34	67.5	67.5	63.1	69.4	67.5	70.1	69.4	69.4	63.7	75.2	73.2	73.2	67.5	63.1	100
LCP 82-89	35	67.5	66.2	61.8	69.4	66.2	68.8	69.4	64.3	62.4	70.1	72.0	72.0	66.2	64.3	82.2

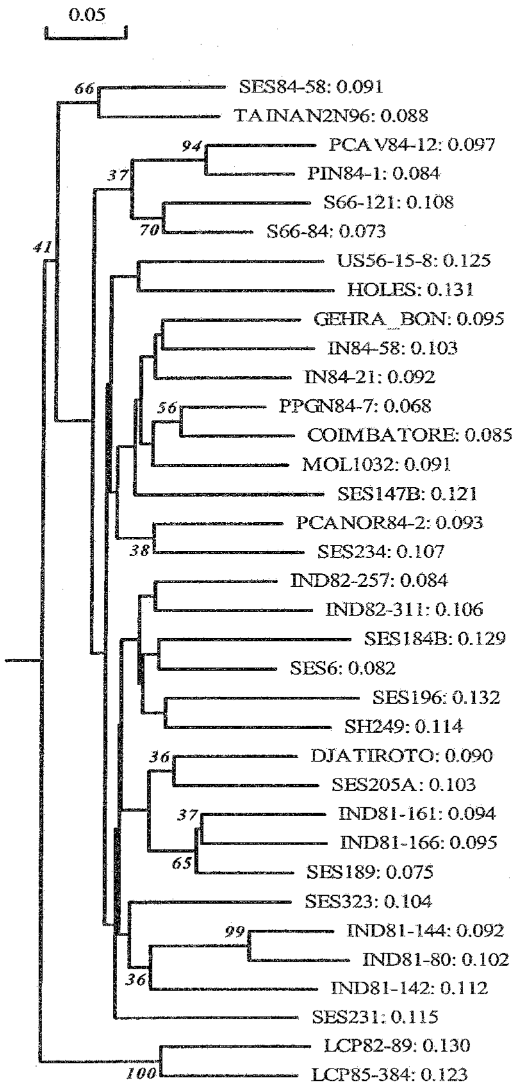

Figure 2. A bootstrapped phylogenetic tree among 33 clones of *Saccharum spontaneum* and two elite sugarcane cultivars LCP 82-89 and LCP 85-384 by DNAMAN® software (Lynnon BioSoft, Vaudreuil, Quebec, Canada) based on the Neighbor-joining algorithm of Saitou and Nei (1987). The graph was set at size (900), branch space (20), and position ($x = 20$; $y = 60$). The bar represents a distance unit of 0.05 when measuring the length of the branches. The number following the name of the sample is its sequence weight. The numerical values on branches are bootstrap or confidence values.

VI, VII and VIII in the neighbor-joining phylogenetic tree, it was related to other clones in the homology tree (not shown). This is due to the fact that Clone SES231 is equal-distantly related to almost all other clones (Table 3).

Discussion

Saccharum spontaneum is cytologically and morphologically diverse (Tai et al. 1995), and generally considered to show greater genetic variability than *S. robustum*, *S. officinarum* (Lu et al. 1994b; Besse et al. 1997), or elite germplasm (*Saccharum* spp. hybrids) (Arceneaux 1967; Harvey and Botha 1996; Harvey et al. 1994). In the present study, genetic similarity coefficients ranged from 60.5 to 88.5% among *S. spontaneum* and elite accessions. This range is narrower than that reported for North American *Saccharum*, Old World *Erianthus*, and elite sugarcane taxa (36–77% in Burner et al. 1997 and 38–99% in Pan et al. 2000). This was not unexpected because clones of North American *Saccharum* and Old World *Erianthus* were more geographically divergent and reproductively isolated than those of *S. spontaneum*. Nonetheless, the results from this study on a limited number of *S. spontaneum* clones support the previous findings that substantial genetic variability had been conserved in *S. spontaneum* (Tai et al. 1995; Lu et al. 1994b).

It is also significant that the 33 *S. spontaneum* clones and two elite cultivars could be distinguished from one another by comparing combined RAPD-PCR fingerprints from two primers, either OPBB-02 and OPBE-04 or OPBB-02 and Primer 262. Primer 262 was previously known to generate substantial polymorphisms in various plant taxa, including sugarcane (Burner et al. 1997; Fritsch et al. 1993). A single RFLP probe (BNL 16.06) was similarly informative for members of *Saccharum* (Lu et al. 1994b). Huckett and Botha (1995) reported that some RAPD bands were inherited in sugarcane families, indicating that RAPD bands could successfully be used as genetic markers. While RAPD-PCR fingerprints have been widely used in diversity studies, the utility of RAPD-based bands as markers is confounded as they may not be locus-specific (reviewed by Besse et al. 1998) and products of different sequences or concentrations can co-migrate with other amplification products (Pan et al. 1997; Pillay and Kenny 1995). To circumvent this problem, Paran and Michelmore (1993) suggested that these putative RPAD markers be cloned and sequenced for PCR primer design.

In this study, the 33 *S. spontaneum* clones were assigned to eight groups based on their RAPD-PCR fingerprints. Grouping appeared to be random with respect to country of origin or morphological and juice traits, as reported by Tai et al. (1995). As expected, the two elite cultivars formed an independent group sharing a genetic similarity coefficient of 82.2%. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed little genetic diversity as well (Arceneaux 1967; Harvey and Botha 1996; Harvey et al. 1994). Harvey and Botha (1996) reported similarities of 77–95% among 20 elite varieties. Similar results were reported by Harvey et al. (1994), who also found that a *S. spontaneum* clone and an elite variety were substantially more divergent (30% similarity). Moreover, Harvey and Botha (1996) cautioned that the extent of diversity among elite and exotic parents tended to decline across generations, presumably because the recurrent parents are genetically similar. We found, however, that LCP 85-384 (Milligan et al. 1994), a BC_4 derivative of US 56-15-8, was about as similar to US 56-15-8 (65.6% homology) as it was to many other *S. spontaneum* accessions (60.5–75.2% homology). Thus, genetic diversity appears to have been conserved in this cross (Lu et al. 1994a). A recent fingerprinting project with microsatellite markers allowed the exploration of elite *Saccharum* germplasm with a narrow genetic base (Pan et al. 2002).

This study should facilitate the use of RAPD-PCR fingerprints in marker-assisted applications in sugarcane breeding. First, the study identified primers that generate substantial polymorphisms among *S. spontaneum* and elite sugarcane germplasm. Similar genetic analysis might also be applicable to other sugarcane-related wild species, such as *S. officinarum* L. (Linnaeus 1753; Grassl 1969), *S. barberi* Jeswiet (Brandes 1958), *S. sinense* Roxb. (Brandes 1958; Roxburgh 1819), *S. robustum* Brandes and Jeswiet ex Grassl (Grassl 1946), *S. edule* Hassk. (Daniels and Roach 1987), and etc. Second, given the resource limitations on conserving clonal germplasm collections, the study demonstrated an approach for identifying and maintaining diverse clones in a *S. spontaneum* core collection. Third, the study demonstrates the potential of specific RAPD-PCR markers for identifying *S. spontaneum* clones and elite cultivars. For example, OPA-11 primed the amplification of four RAPD-PCR products from the current Louisiana leading variety LCP 85-384 but none from the *S. spontaneum* clone Djatiroto. This fingerprint has been reproduced routinely from LCP 85-384 in our laboratory. The second largest RAPD product of OPA-11 had an approximate size of 366 bp and appeared to be cultivar-specific. This marker, designated as OPA-11-366, has been used in the local breeding program to assist in the selection of interspecific hybrids between the *S. spontaneum* Clone Djatiroto and two sugarcane cultivars, LCP 85-384 and CP 62-258 (Pan and Burner, unpublished).

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