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Genome size variation in three *Saccharum* species

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Abstract *Saccharum* species are autopolyploids with ploidy level ranging from 5× to 16*x*, and are considered the most complex genomes among crop plants. In present study, the genome sizes of 28 *Saccharum spontaneum* accessions, 15 *Saccharum officinarum* accessions, 28 *Saccharum robustum* accessions, and 12 *Saccharum* hybrids spp. were analyzed using flow cytometry. The estimated genome sizes of *S. officinarum* accessions ranged from 7.50 to 8.55 Gb with an average size of 7.88 Gb. In *S. robustum*, the estimated genome sizes ranged from 7.65 to 11.78, reflecting the variation of ploidy level. In *S. spontaneum*, the estimated genome sizes varied widely, with a range from 3.36 to 12.64 Gb, also due to variation of ploidy level. The average monoploid genome size of *S. officinarum* was 985 Mb, and that of *S. spontaneum* was 843 Mb. The results also showed that genome sizes were correlated with chromosome numbers, and based which, that the unknown chromosome numbers of some accessions could be predicted. The estimated genome sizes of *Saccharum* germplasm also helped identify some mislabeled accessions and yielded information critical for sugarcane breeding and genome sequencing programs.

Keywords DNA content · Genome size · Flow cytometry · Sugarcane · Saccharum species

Introduction

Sugarcane (*Saccharum* spp., Poaceae), is a large perennial crop mostly grown in the tropical and

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subtropical areas worldwide and accounts for 70% of world sugar production. It is one of most efficient crops in converting solar energy into chemical energy. Sugarcane is a first generation biofuel crop used for ethanol production as an alternative source of energy (Lam et al. 2009), and has proven to be an efficient feedstock for generating biofuel.

Sugarcane belongs to the genus *Saccharum*, which includes *Saccharum spontaneum*, *Saccharum robustum*, *Saccharum officinarum*, *Saccharum barberi*, *Saccharum sinense,* and *Saccharum edule* based on conventional taxonomy. *S. spontaneum* and *S. robustum* are wild species with basic chromosome number $x = 8$ and $x = 10$, respectively (D'Hont et al. 1996, 1998; Ha et al. 1999). *S. officinarum* is a domesticated sugar-producing species with auto-octoploid ($2n = 8x = 80$), and is likely derived from the wild autopolyploid species *S. robustum* (mainly $2n = 60, 80$) as shown by the close relationship of these two species in DNA marker studies (D'Hont et al. 1993; Lu et al. 1994; Schenck et al. 2004). The other three species are hybrids. *S. barberi* and *S. sinense* are interspecific hybrids between *S. officinarum* and *S. spontaneum* (D'Hont et al. 2002), *S. edule* could be an interspecific or intergeneric hybrid between either *S. officinarum* or *S. robustum* with a species in the *Saccharum* complex (Daniels and Roach 1987). Modern sugarcane varieties have been cultivated since these varieties were bred about a century ago by interspecific crosses between *S. officinarum* and *S. spontaneum* and backcross to *S. officinarum*. For this reason, our study of genome size variation was focused on these three species, namely, *S. officinarum, S. robustum* and *S. spontaneum.*

The ploidy level and chromosome numbers vary among accessions of *Saccharum* species with the exception of *S. offcinarum* ($2n = 80$). For *S. robustum*, the chromosome number range from $2n = 60$–170, and about 35% accessions have $2n = 80$ chromosomes (Irvine 1999). The chromosome numbers of *S. spontaneum* accessions range from $2n = 36$–128, and 77% of its accessions have chromosome numbers equaling multiples of eight (Irvine 1999).

These variations at both ploidy and chromosome number level have resulted in different genome sizes among accessions of *Saccharum* species and *Saccharum* hybrids. Previously, the genome sizes of a few reported (1–3) accessions of four *Saccharum* species were estimated to range from 5.28 to 8.67 Gb (Arumuganathan and Earle 1991). For the cultivar R570, the genome size was estimated to be about 10 Gb with ploidy level about $12x$ (Tompkins et al. 1999; D'Hont 2005). In the analysis of genome sizes for three sugarcane interspecific F1 families, obtained from *S. officinarum* cv. Green German × *S. spontaneum* cv. IND 81-146, *spontaneum* cv. Pin 84-1 × *S. officinarum* cv. Muntok Java (PM), and Pin 84-1 × CP 70-1133 (PCP) crosses, 2C DNA contents ranged from 3.05 pg for IND 81-146 to 8.91 pg for Muntok Java, and the DNA contents of the progeny were within the range of DNA values of their respective parents (Edme et al. 2005).

In contrast to the studies on ploidy and chromosome numbers, little is known about the variation of genome size within and among *Saccharum* species. Knowing the genome sizes of various *Saccharum* species may help in the utilization of sugarcane genetic resources for breeding program in two ways: (1) validating the ploidy level for accessions with chromosome numbers; (2) predict ploidy level for those without chromosome numbers. Recently, the sugarcane genomic community has laid out sugarcane genome sequencing strategies (Souza et al. 2011). With the rapid advance of sequencing technologies, sequencing and re-sequencing sugarcane genome will become a reality in the near future. The estimates of genome sizes of *Saccharum* species will provide essential information for selecting accessions for sugarcane genome sequencing and re-sequencing projects. In present study, a number of accessions from the three original *Saccharum* species, namely, *S. officinarum*, *S. spontaneum,* and *S. robustum*, were selected to estimate the genome sizes using flow cytometry. Our objectives were: (1) To assess the degree of genome size variation within and among *Saccharum* species; (2) To predict chromosome number based on the genome sizes for those accessions without chromosome counts; and (3) To estimate the monoploid genome size for *Saccharum* species.

Materials and methods

Plant materials

Leaf tissue from 16 accessions of *S. officinarum* (maintained in Maunawili Station, Oahu, Hawaii), 28 of *S. robustum* (maintained in USDA World Collection-Miami), 28 accessions of *S. spontaneum* (maintained in Maunawili Station, Oahu, Hawaii, Houma,

Louisianna, Weslaco, Texas, and Canal Point, Florida), and 12 *Saccharum* hybrid spp. (maintained in Maunawili Station, Oahu, Hawaii and Canal Point, Florida) were used for analyzing nuclear DNA content (Table 1). Of the *Saccharum* hybrids spp., 10 are F1 plants obtained from a segregating population derived from a cross *S. officinarum* cv. LA Purple × *S. robustum* Molokai5829. About 1 g of young leaf tissues was collected from each accession was used for flow cytometry analysis.

Flow cytometry analysis

The protocol for flow cytometry analysis was modified from the procedure by Arumuganathan and Earle (1991).The nuclei from Chicken Red blood cells (2.5 pg/2C, Glycine max (2.45 pg/2C), Oryza sativa cv Nipponbare (0.96 pg/2C), Arabidopaia thaliana (0.36 pg/2C) were used as the internal standard. Fifty milligram (mg) fresh leaf tissues of the Saccharum species and 25 mg standard were placed on ice in a sterile 35 × 10 mm plastic petri dishes. The tissues from individual samples were chopped into 0.25 mm to 1 mm segments in a solution containing 50 mM KCl,10 mM MgSO4.7H2O, 3 mM dithiothreitol, 5 mM Hepes, 0.1 mg/mL propidium iodide, 1.5 mg/mL DNase free RNase (Rhoche, Indionapolis, IN), 0.25% Triton X-100 and pH 8.0. The suspended nuclei were extracted by a pipettor and filtered with 30-μm nylon mesh. Following filtration, samples were incubated for 30 min at 37 °C before flow cytometric analysis. Suspensions of sample nuclei were combined with suspension of standard nuclei (prepared in above solution) and analyzed by a FACScalibur flow cytometer (Becton–Dickinson, San Jose, CA).

For each accession, four replications (one leaf per replication) were analyzed. The propidium iodide fluorescence area signals (FL2-A) of 1,000 nuclei per measurement were collected to analyzed by CellQuest software(Becton–Dickinson, San Jose, CA) on FACSCalibur flow cytometer (Becton–Dickinson, San Jose, CA). The mean position of the G0/G1 (Nuclei) peak of the sample and the internal standard were determined by Cell Quest software. The genome size (in megabase pairs, Mbp) was estimated according to the formulae by Lysak and Dolezel (1998) with conversion of 1 pg = 980Mbp (Dolezel et al. 2003).

Results

The genome size of *Saccharum* species and *Saccharum* Spp. hybrid

The domesticated *S. officinarum* is defined by high sugar content and constant chromosome number

Table 1 The estimated genome sizes of the *S. officinarum* accessions

No.	Accession #	DNA content (pg/2C)	St. dev (±)	Estimated genome size (Gb/2C)	Estimated Mb/haploid set	Estimated chr no (2*n*)
1	Akoki	8.27	0.05	8.09	1011.0	80
2	Halalii	8.13	0.03	7.95	993.9	80
3	HC63	8.74	0.20	8.55	1068.5	80
4	Honuaula	8.21	0.27	8.03	1003.7	80
5	Kokea	11.98	0.37	11.72	976.4	120
6	Laukona	7.67	0.11	7.50	937.7	80
7	Lauloa	8.28	0.22	8.10	1012.2	80
8	Lehu	8.56	0.06	8.37	1046.5	80
9	Mahaiula	7.74	0.13	7.57	946.2	80
10	Manulele	7.89	0.06	7.72	964.6	80
11	Moano	8.05	0.09	7.87	984.1	80
12	Pakaweli	8.13	0.09	7.95	993.9	80
13	Uahiapele	7.68	0.17	7.51	938.9	80
14	Waiohia	7.82	0.11	7.65	956.0	80
15	Yellow Caledonia	7.85	0.10	7.68	959.7	80
16	LA Purple	7.83	0.08	7.66	957.2	80

Table 2 The estimated genome sizes of the *S. robustum* accessions

No.	Accession	DNA content (pg/2C)	St. dev (±)	Estimated genome size (Gb/2C)	Estimated chr no (2*n*)
1	Mol6081	8.73	0.42	8.54	80
2	Mol6136	11.69	0.38	11.43	120
3	NG 77-084	7.82	0.06	7.65	80
4	NG 77-083	7.87	0.06	7.70	80
5	IM 76-228	9.05	0.06	8.85	80
6	IN 84-076	9.13	0.11	8.93	80
7	IM 76-232	9.20	0.06	9.00	80
8	IS 76-184	9.22	0.08	9.02	80
9	Tolo Fua Lau I	9.50	0.41	9.29	80
10	IJ 76-496	10.13	0.10	9.91	
11	MOL 6077	10.25	0.06	10.02	
12	NH 70-015	10.27	0.12	10.04	
13	Tolo Fua Lau I	10.33	0.05	10.10	
14	IJ 76-412	10.36	0.08	10.13	
15	NG 57-055	10.82	0.22	10.58	
16	IJ76-482	10.98	0.13	10.74	
17	MOL 6091	11.13	0.10	10.89	
18	NG 57-012	11.24	0.11	10.99	
19	IJ 76-547	11.28	0.23	11.03	
20	IN 84-052	11.33	0.16	11.08	
21	NG 77-107	11.33	0.14	11.08	
22	IN 84-050	11.41	0.13	11.16	
23	Fiji 1	11.49	0.05	11.24	
24	NG 57-024	11.91	0.21	11.65	120
25	IJ 76-435	11.96	0.10	11.70	120
26	IJ 76-424	12.00	0.12	11.74	120
27	NG 77-196	12.01	0.16	11.75	120
28	IN 84-045	12.05	0.11	11.78	120

$2n = 80$. Hawaiian canes are *S. officinarum* clones that were brought to the islands and cultivated by the native polynesians. The estimated genome sizes of 16 Hawaiian canes are the mostly consistent with only one exception, "Kokea". Fifteen of the 16 Hawaiian cane accessions have genome sizes ranged from 7.50 to 8.55 Gb/2C with average size 7.88 Gb (Table 1). The outlier clone, Kokea, has an estimated genome size of 11.72 Gb, which is 48.7% larger than the average genome size of *S. officinarum*.

The accessions of *S. robustum* are known to vary on ploidy levels and chromosome numbers. Among the 28 accessions maintained in the USDA sugarcane germplasm repository in Miami, FL, USA, the estimated genome sizes range from 7.65 to 11.78 Gb (Table 2). No chromosome information is available for these 28 accessions. The distribution of genome sizes among *S. robustum* accessions is shown in Fig. 1.

Most *S. spontaneum* accessions have chromosome counts available, ranging from $2n = 4x = 32$ (another culture derived haploid) to $2n = 16x = 128$ (Moore 1989). The estimated genome sizes of the *S. spontanum* accessions mostly reflect their chromosomes counts, ranging from 3.36 to 12.64 Gb (Table 3). However, the genome sizes of several *S. spontanum* accessions differ from the expected genome sizes based on their chromosome numbers. The most noticeable is a haploid (tetraploid) clone of SES208, namely, AP85-68 ($2n = 32$), with a genome size of 6.62 Gb, about twice the size of another haploid clone of SES208, AP85-441 ($2n = 32$) at 3.36 Gb, but similar to the genome sizes of double haploid clone

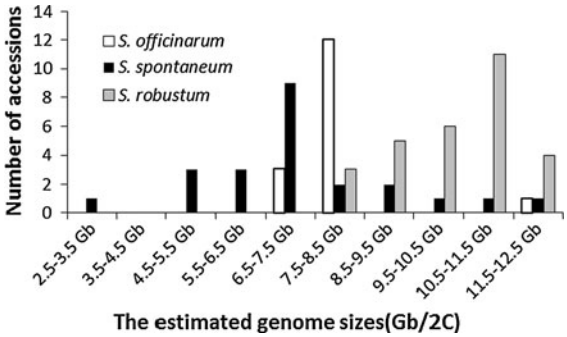

Fig. 1 The distribution of estimated genome sizes of *S. spontaneum, S. officinarum,* and *S. robustum* accessions

AP83-108 ($2n = 2 \times 32 = 64$) of SES208 at 6.32 Gb and the octoploid SES208 itself ($2n = 64$) at 6.74 Gb. This haploid clone AP85-68 is likely a double haploid or an octoploid with $2n = 64$. Using estimated monoploid genome size (see section below), the chromosome numbers of five *S. spontaneum* clones were corrected, including SES113A, US48-61, SES561, US56-15-8, and SES234 (Table 3). The distribution of genome sizes among *S. spontaneum* accessions is shown in Fig. 1.

Based on chromosome number information of *S. spontaneum*, the correlation between chromosome number and genome size was analyzed, and these two variables were positively correlated with $R^2 = 0.925$ (Fig. 2).

Of the 14 *Saccharum* hybrids spp. accessions, 10 are F_1 progeny obtained from *S. officinarum* cv. LA Purple ($2n = 80$) × *S. robustum* Molokai 5829 ($2n = 80$). This is one of the rare crosses in *Saccharum* spp. with a balanced transmission of 40 chromosomes from each parent. The F_1 progeny are expected to have $2n = 80$ chromosomes and similar genome sizes. Nine of the 10 F_1 progeny have similar genome size, ranged from 7.02 to 8.21 Gb with the average at 7.63 Gb, indicating that they are likely to have $2n = 80$ chromosomes. The one outlier progeny, 91-9049, with a genome size of 10.36 Gb, could be product of an unreduced gamete resulting in somatic chromosome number of $2n = 120$. The genome size of one parent LA Purple is 7.66 Gb. The male parent, Molokai 5829 was not available for this study since it was accidentally lost during a field clearing operation. Its genome size was similar to that of LA Purple, because the average genome size of nine F_1 progeny is similar to that of LA Purple at 7.66 Gb.

The genome sizes of two *Saccharum* hybrid spp., namely, Green German 1# and Montok Java 1# were 11.41 and 13.63 Gb, respectively, which correlate with their chromosome numbers (Table 4).

The monoploid genome size of *Saccharum* species

Estimating the monoploid genome size would provide crucial information for sequencing the genomes of these autopolyploid species. The monoploid genome size of each accession that had a known chromosome number was estimated by genome size/chromosome number/basic chromosome number. The monoploid genome size of *S. officinarum* ranged from 937.7 to 1068.5 Mb with an average of 984.9 Mb (Tables 1, 5). The monoploid genome size of *S. spontaneum* ranged from 732.5 to 985.5 Mb with an average of 843.1 Mb (Tables 3, 5). No chromosome information is available for *S. robustum*, however, its monoploid genome size was found to be similar to that of *S. officinarum* at 984.9 Mb, based on the analysis of genome size estimates of the F_1 individuals described above.

Discussion

The autopolyploid *Saccharum* species varies in chromosome number and ploidy level, which are critical information for finding the optimal combination of parental materials to make crosses in sugarcane and energy cane breeding programs. However, chromosome counting is a laborious process and a few labs are doing it. For those well characterized breeding materials, mislabeling of *Saccharum* accessions often occurs during germplasm exchange or replanting. Genome size estimation using flow cytometry provides an alternative approach to either verify chromosome information if available or estimate ploidy level if chromosome information are not available. Our results revealed that a haploid *S. spontaneum* clone is actually a double tetraploid or octoploid. In addition, the chromosome numbers were corrected for five *S. spontaneum* accessions, one *S. officinarum* accession, and one F_1 progeny, further supporting the usefulness of genome size estimates.

Modern sugarcane cultivars (*Saccharum* hybrids spp.) are interspecific hybrids mostly of *S. officinarum* and *S. spontaneum*, and to a much lesser extent, of *S. robustum*, was extensively used in the breeding

Table 3 The estimated genome sizes of the *S. spontaneum* accessions

No.	Accession #	Chr. no (2*n*).	DNA content pg/2C	St. dev (±)	Estimated genome size(Gb/2C)	Estimated Mb/ haploid set	Estimated chr no (2*n*)
Maintained in Hawaii							
1	SES208 haploid (AP85-68)	32	6.77	0.05	6.62	827.6	64*
2	SES208 haploid (AP85-441)	32	3.44	0.05	3.36	841.1	
3	SES208 double haploid (AP83-108)	2 × 32	6.46	0.10	6.32	789.7	
4	SES208	64	6.89	0.13	6.74	842.3	
5	SES113A	48	7.85	0.47	7.68	767.7	80*
6	SES186	52	6.55	0.07	6.41	985.5	
7	SES239/43	72	7.31	0.05	7.15	794.4	
8	SES561	128	7.54	0.32	7.37	737.4	80*
9	US56-14-4	80	7.49	0.32	7.33	732.5	
10	US48-61	102	6.76	0.10	6.61	826.4	64*
Maintained in Louisianna							
11	US56-15-8	80	6.81	0.29	6.66	832.5	64*
12	SES234	64	8.89	0.16	8.69	869.4	80*
13	Spont17	48	5.36	0.05	5.24	873.7	
14	Spont24	48	5.41	0.05	5.29	881.8	
15	Tainan	96	10.13	0.21	9.91	825.6	
16	Djatiroto	58	6.64	0.10	6.49	895.7	
Maintained in Weslaco, Texas							
21	Okinawa #13		12.92	0.09	12.64	789.7	128
22	PCA NOR 84-04		8.97	0.03	8.77	877.3	80
23	S. spont #11		7.16	0.03	7.00	875.3	64
24	Dacca		7.63	0.07	7.46	932.8	64
25	Djantoer II (2)		11.51	0.20	11.26	938.1	
Maintained in Florida							
28	IND 81-146	52–56	5.24	0.05	5.12	732.1	
29	PIN 84-1	96	8.28	0.48	8.10	810.0	80*

The chromosomes numbers analysis were furnished by Rao (1983)

* The chromosome numbers were corrected based on the estimated genome sizes

program, particularly in Hawaii. We estimated the genome sizes of a combined 72 accessions of these species from four sugarcane growing states of the U.S., namely, Florida, Hawaii, Louisiana, and Texas. The estimated or corrected ploidy level would help the breeders to design their crosses. We also estimated the monoploid genome size for *S. officinarum* and *S. spontaneum,* thus providing the basic information for sequencing the sugarcane genomes (Souza et al. 2011).

Modern sugarcane and its ancestors (*Saccharum* species) are considered to be the most genetically complex crop. The evidence from analysis of *Adh1* gene and BAC sequences of sugarcane species showed that sugarcane is a recently evolved autopolyploid with two rounds of genome wide duplications that occurred 1–2 million years ago, with divergence between the *S. officinarum* and *S. spotaneum* occurring 1.5–2 million years ago (Jannoo et al. 2007). In present study, the genome size of *S. officinarum* was stable (Table 1), which was in agreement with former result that more than 90% of *S. officinarum* accessions had chromosome counts of $2n = 80$ (Irvine 1999), and those with chromosome numbers other than $2n = 80$ would have been considered mis-identification by either mislabeling or misclassification. In contrast, the genome size of *S. spontaneum* varies between accessions, reflecting the wide range of chromosome

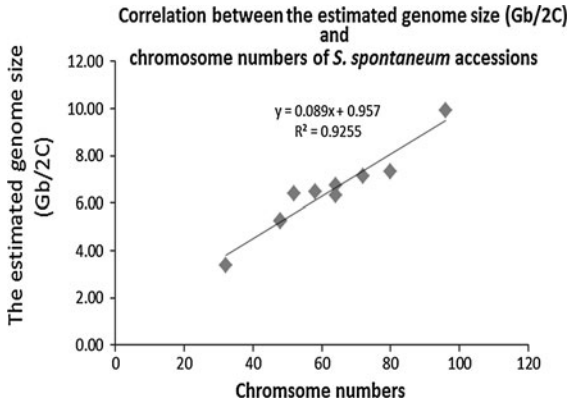

Fig. 2 Correlation between the estimated genome size (Gb/2C) and chromosome numbers of *S. spontaneum* accessions

number variations in this species (Irvine 1999). The variation of genome sizes among *S. spontanum* can be explained by distinctively greater diversity within *S. spontaneum* compared to other *Saccharum* species, which was demonstrated by RFLP (Burnquist et al. 1992; Lu et al. 1994; Nair et al. 1999), AFLP (Selvi et al. 2006), randomly amplified polymorphic DNA (RAPD) (Nair et al. 1999; Pan et al. 2004), and simple sequence repeat (SSR) markers (Selvi et al. 2003).

S. robustum is thought to be the ancestral species of *S. officinarum* (Brandes 1956; Sreenivasan et al. 1987; Daniels and Roach 1987; Grivet et al. 2006). Our results showed that the monoploid genome size of *S. robustum* is about the same as that of *S. officinarum* from the analysis of F_1 individuals from a cross between these two species using two varieties with the same $2n = 80$ chromosomes. The genome sizes of *S. robustum* accessions varied, mostly larger than that of octoploid genome, reflecting the increased ploidy levels of these accessions. However, the range of genome sizes of *S. robustum* is not as wide as that of *S. spontaneum*, reinforcing the conclusion of greater genetic diversity in *S. spontaneum* discussed above.

Table 5 The estimation for monoploid of *S. officinarum* and *S. spontaneum*

Species	N	Mean(Mb)	Std. error
S. officinarum	15	984.9	10.05
S. spontaneum	10	843.1	21.03

The genetic diversity for native Hawaiian *S. officinarum* accessions was studied previously using restriction fragment length polymorphism (Schenck et al. 2004). The outlier Kokea with much large genome size was grouped with five commercial hybrids, and our results further confirmed that Kokea is a mislabeled commercial hybrid, not a Hawaiian cane. In that study, Akoki, HC63, Lauloa and Pakaweli were grouped in Cluster I, whereas Manulele, Waiohia and Yellow Caledonia were grouped into Cluster V (Schenck et al. 2004). The estimation of genome sizes of the accessions had a range from 7.95 to 8.55 Gb/2C (Cluster I) and from 7.65 to 7.72 Gb/2C (Cluster V), respectively,

Table 4 The estimated genome sizes of the *Saccarhum* hybrid accessions

No.	Accession #	Chr. no (2*n*).	DNA content pg/2C	St. dev ±	Estimated genome size (Gb/2C)	Estimated chr no (2*n*)
The F1 families of LA Purple × Mol 5829, maintained in Hawaii						
1	91-9144		7.31	0.17	7.15	
2	91-9121		7.86	0.11	7.69	
3	91-9100		7.18	0.08	7.02	
4	91-9085		7.34	0.13	7.18	80
5	91-9074		8.25	0.13	8.07	80
6	91-9064		8.39	0.24	8.21	80
7	91-9049		10.59	0.09	10.36	120
8	91-9040		8.02	0.10	7.84	80
9	91-9027		8.00	0.12	7.82	80
10	91-9016		7.86	0.31	7.69	80
Maintained in Florida						
11	Green German 1#	97–117	11.67	0.26	11.41	
12	Montok Java 1#	140	13.94	0.58	13.63	

showing a clear difference between these two clusters of Hawaiian cane accessions.

In present study, the average monoploid of the *S. officinarum* accessions and *S. spontaneum* were estimated to be 984.9 and 843.1 Mb, respectively. These results were higher than previous estimations of 930 Mb for *S. officinarum* and 750 Mb for *S. spontaneum* (Arumuganathan and Earle 1991). As a result from the high variation of genome sizes among the *Saccharum* species, an estimate based on only a few accessions may cause a statistical bias. The monoploid genome size might indeed vary among accessions. For this reason, it would be necessary to estimate the monoploid genome size of any accession once subject to genome sequencing. Our results support the view that the sugarcane monoploid size is similar to that of the sorghum haploid genome (D'Hont and Glaszmann 2001).

The analysis of genome size can be used for surveying polyploidy, intergenic hybridization, and intraspecific diversity (Doleze 1997). The genome sizes and chromosome numbers were discovered to correlate significantly between buffalograss (Buchloë dactyloides) (Johnson et al. 1998), fine fescues (*Festuca* spp.) (Huff and Palazzo 1998), Agave tequiliana (Palomino et al. 2003) and switchgrass (Costich et al. 2010). In a study of F_1 progeny from *S. officinarum* and *S. spontaneum*, genome sizes were highly correlated with chromosome numbers on the basis of five accessions of *Saccharum* spp. (Edme et al. 2005). In present study, 10 accessions of *Saccharum* spp. with known chromosome numbers were used for analyzing the correlation between genome size and chromosome number. The results showed that genome sizes were correlated with chromosome number (Fig. 2). For this reason, the clone with the lowest chromosome number, haploid (tetraploid) clone AP85-441 ($2n = 32$), would be a good candidate for sequencing the sugarcane genome.

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