

DETERMINATION OF DNA CONTENT AND GENOME SIZE IN SUGARCANE

S.J. Edmé*, J.C. Comstock, J.D. Miller, and P.Y.P. Tai

USDA-ARS Sugarcane Field Station, 12990 US Hwy 441 N., Canal Point, FL 33438

*Corresponding author: sedme@saa.ars.usda.gov

ABSTRACT

An accurate determination of the progeny types in sugarcane (*Saccharum* spp.) crosses would facilitate genetic and genomic analyses. Flow cytometry analysis was performed on three sugarcane interspecific F_1 families, obtained from *S. officinarum* cv. Green German x *S. spontaneum* cv. IND 81-146 (GI), *S. spontaneum* cv. Pin 84-1 x *S. officinarum* cv. Muntok Java (PM), and Pin 84-1 x CP 70-1133 (PCP) crosses, to measure variation of the nuclear-2C DNA content and genome size and to identify putative $2n + n$ hybrid progeny. Nuclei were isolated from young leaves and stained with 4', 6-diamidino-2-phenylindole (DAPI). Fluorescence ratios, calculated relative to maize (*Zea mays* cv. CE-777) which was used as an internal standard, were converted to DNA content values (picograms-pg and megabase pairs-Mbp) and analyzed. A 2.92-fold difference in 2C-DNA content was observed among the parents, ranging from 3.05 pg (IND 81-146) to 8.91 pg (Muntok Java) for a 1474 to 4298 Mbp genome size. DNA contents of the progeny were within the range of DNA values calculated for their respective parents, implying chromosome transmission was according to an $n + n$ mechanism. Variation was largest (40 %) in the GI hybrids which included four progeny with putative $2n + n$ transmission and lowest (18 and 16 %) in the PM and PCP hybrids. The PM group presented progeny with the largest mean genome size (7.15 pg), followed by the PCP (6.27 pg) and GI (5.67 pg) families. Only small variation existed among the commercial cultivars, indicating that genome size is stable for this group. Correlations between genome size and phenotypic traits were in general low, ranging from $r = 0.30$ to $r = -0.49$, but significant ($P < 0.001$) in this set of interspecific hybrids. However, the relationship between chromosome number and genome size was highly significant ($P < 0.001$). Flow cytometry can be an effective tool to assess hybridization in interspecific crosses, to identify $2n + n$ clones, and to determine chromosome number in sugarcane.

INTRODUCTION

Species within the genus *Saccharum* are well delineated by cytogenetics and well characterized by morphological characters (Price, 1960; Bremer, 1961; Roach, 1969; Nair, 1975; Daniels and Roach, 1987; Sreenivasan et al., 1987). Variation in chromosome numbers ranges from 40 (*S. spontaneum*) to 130 (interspecific hybrids) with a basic number set at 8 to 10 (d'Hont et al., 1998). Commercial sugarcane cultivars are complex allopolyploids or interspecific hybrids that originated from crosses between a few parents belonging mostly to the *S. officinarum* and *S. spontaneum* species (Sreenivasan et al., 1987). However, little information is available on the nuclear genome size and DNA content in sugarcane. Such information can be valuable in understanding the cytogenetic phenomena in wide crosses (Burner, 1997) and complement conventional and molecular germplasm development programs aimed at increasing genetic diversity and gene exchange.

Flow cytometry is a simple and efficient technique that is used more commonly to determine ploidy levels (De Laat et al., 1987), nuclear DNA content, genome size, and hybrid verification in plants (Arumuganathan and Earle, 1991b; Bennett and Leitch, 1995; Doletzel, 1997). Cytometric determination of nuclear DNA content has proved to be useful in studying the variation in interspecific and intraspecific DNA content (Dolezel et al., 1989; Hammat et al., 1991) in plants. Several studies on variation in DNA content in polyploid populations (Kenton et al., 1986) showed a decrease in DNA amount in subsequent generations.

The benefits of this technique have not been exploited yet in sugarcane. Arumuganathan and Earle (1991a) is the only report to list the DNA content (2C) and genome size (1C) of four *Saccharum* species along with those of other plant species. DNA content (pg) and genome size (Mbp) were reported as 5.28-7.47 pg/2547-3605 Mbp for *S. officinarum*, 6.54-8.54 pg/3156-4121 Mbp for *S. barberi*, 6.53 pg/3151 Mbp for *S. robustum*, and 8.67 pg/4183 Mbp for *S. sinense*. With an increased interest in exploiting the wild relatives of sugarcane to increase genetic variability and diversity, there are many interrelated issues, linked to interspecific hybridization in sugarcane, that can be addressed by flow cytometry, viz., the existence of a polyploid (5x to 14x) series (Sreenivasan et al., 1987; Burner and Legendre, 1994), chromosome pairing (Burner and Legendre, 1993), genic imbalance and endosperm degeneration (Berding and Roach, 1987), and identification of $2n + n$ progeny (Burner, 1997).

A set of genetically distinct interspecific populations, developed by Dr. Peter Tai at the USDA-ARS Canal Point Station (FL), are being used for molecular studies (marker screening, QTL searching, and gene mapping). Flow cytometry analyses are being introduced into the program to gain more insight into the variation and transmission of DNA content within these populations. Information on genome size and DNA content has practical significance for molecular characterization of populations with regard to the choice of primers and restriction enzymes, the construction of chromosome-specific libraries, and gene mapping (Cotter et al., 1989; Van Dilla and Deaven, 1990; Bennett et al., 2000). The sugarcane interspecific hybrids under study encompass progeny of crosses that contain various genomic combinations. Three hybrid progeny from two of these populations, that were selected by Burner (1997) as putative $2n + n$ clones based on stalk diameter, turned out to have arisen from $n + n$ transmission. Identification of clones derived from self-pollination and of $2n + n$ hybrid clones from interspecific crosses is important to sugarcane breeders who usually rely unsuccessfully on phenotypic traits, such as stalk diameter, to identify hybrid progeny. Sugarcane breeders assume that an increase in DNA content in $2n + n$ hybrids would be accompanied with an increase in cane and sugar yields (Burner, 1997). The objectives of this study were to estimate nuclear DNA content and genome size in a set of interspecific sugarcane hybrids and their parents, to measure the variation in these populations, and to identify putative $2n + n$ progeny.

MATERIALS AND METHODS

Plant materials

Three F_1 interspecific populations were developed from three crosses involving two *S. officinarum* (Green German and Muntok Java), two *S. spontaneum* (IND 81-146 and Pin 84-1), and the commercial cultivar, CP 70-1133 (Table 1). Green German and Muntok Java were used as female and male parents, respectively, in the cross with a *S. spontaneum*. The identities of

both Green German and Muntok Java as true *S. officinarum* species were questioned by Burner (1997) who identified them as putative hybrids after counting their chromosomes. IND 81-146 was the pollen parent in the cross with Green German. Pin 84-1 was used as the female parent in the crosses with Muntok Java and CP 70-1133. Six other Florida commercial cultivars (CP 72-1210, CP 72-2086, CP 80-1743, CP 80-1827, CP 81-1254, and CP 89-2143), three other *S. officinarum* (Black Cheribon, Louisiana Purple, and Oi Dang), and an interspecific hybrid cultivar from Coimbatore, India (NCo 310) were included in the analysis. Black Cheribon is one of the earlier progenitors of modern sugarcane cultivars and along with Louisiana Purple was grown commercially prior to the release of hybrid genotypes for commercial production. Parents, hybrids, and commercial cultivars were planted at Canal Point on Hatton's farm on 14 Feb. 2003 in 2 m-long single row-plots with no replications. The soil type of this field was a Torrey muck (organic), classified as euic hyperthermic typic haplosaprist. NCo 310 and the other three *S. officinarum* species, with known chromosome numbers, were planted in a separate nursery at the Canal Point station.

Isolation and staining of nuclei

Cell nuclei were isolated from young leaves of the field-grown plants. Multiple samples were taken on different plants from each plot. The internal soft tissues from the leaf whorl were used. Approximately 0.5 cm² of leaf tissue from each sample and greenhouse-grown *Zea mays* cv. CE-777 (2C DNA content = 5.43 pg; Lysak and Dolezel, 1998) plants, used as an internal standard, were simultaneously chopped in a petri dish with a sharp razor blade in 0.5 ml buffer (High resolution DNA kit, Partec GmbH-Munster, Germany¹). Seeds of the standard were provided by Dr. Jaroslav Dolezel, Institute of Experimental Botany, Czech Republic. Nuclei suspensions were passed through a 40-µm nylon filter. Staining solution (600 ml), containing 4', 6-diamidino-2-phenylindole (DAPI), was added to the filtered suspensions. Nuclei were analyzed immediately using a Partec-PA flow cytometer (Partec GmbH-Munster, Germany) equipped with a mercury arc lamp and fluorescence intensities were registered over 500 channels and displayed as histograms. Prior to the analysis, linearity of the instrument was checked and the gain was adjusted by gating at channel 50 and locating the G₀/G₁ peak of the internal standard at channel 100. The G₀/G₁ phase (2C DNA content) corresponds to the first period of cell growth in the cell cycle, followed by the S phase, then the G₂ phase, a second period of cell growth (4C DNA content), and finally by mitosis (2C DNA content). At least 10,000 nuclei were analyzed per run and each sample was repeated two to four times. Fluorescence ratios, relative to the standard, were used to calculate DNA content (in picograms, pg) and genome size (in megabase pairs, Mbp) according to the following formulae (Lysak and Dolezel, 1998):

$$2C \text{ DNA content/sample (pg)} = \frac{[\text{Sample Peak mean} \times \text{Standard DNA content (}=5.43 \text{ pg)}]}{\text{Standard Peak mean}}$$

$$1C \text{ Genome size (Mbp)} = \frac{[\text{Sample 2C DNA content (pg)} \times 965 \text{ Mbp}]}{2}$$

¹ Product names and trademarks are mentioned to report factually on available data; however, USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA does not imply approval of the product to the exclusion of others that may also be suitable. The experiments reported comply with the current laws of U.S.A.

The symbol C corresponds to the haploid nuclear genome size, i.e. the DNA content of the haploid set of chromosomes, with 1 pg = 965 Mbp (Bennett and Smith, 1976).

DAPI is an AT-specific fluorochrome that tends to overestimate genome size in plants (Dolezel et al., 1992). Leaf samples of radish (*Raphanus sativus* cv. Saxa; Dolezel et al., 1992), soybean (*Glycine max* cv. Polanka; Dolezel et al., 1994), and tomato (*Lycopersicon esculentum* cv. Stupicke polni; Dolezel et al., 1992), with known DNA content and genome size (seeds were provided by Dr. Dolezel), were co-chopped and run with the standard (corn cv. CE-777) on the same instrument settings. A mean correction factor of 1.85 was estimated from the ratios of calculated DNA content and genome size for these plants to their known values. This factor was used to correct the values obtained for the sugarcane samples.

Data analyses

Statistical analyses were performed with SAS 9.0 (SAS Institute Inc., 2002), using a one-way ANOVA on the DNA content values measured from two to four leaf samples with genotypes nested within groups. Differences within and between groups of progeny and parents were declared significant using honestly significant differences at $P < 0.05$ by Tukey's multiple range test (Steel and Torrie, 1980). Spearman correlation analyses were carried out to account for cases of non-normality in the data and evaluate the existence of possible relationships within the hybrid populations between DNA content and other quantitative characters (stalk height, diameter and weight, Brix, pol, and sucrose content). Linear regression analysis was performed to define the relationship between DNA content and chromosome number for the six genotypes with reported counts (Burner, 1997). The resulting equation was used to predict chromosome numbers for the other clones. Bivariate density plots were generated to gain more insight into the relationship between genome size and each of the phenotypic traits. Since all plots appeared to be relatively similar, only one figure for the genome size-Brix relationship was displayed.

RESULTS

Relative nuclear DNA content

Flow cytometric analyses of nuclei isolated from sugarcane leaves show one peak that corresponds to the G₀/G₁ phase (2C level) of the cell cycle (Figure 1). Peaks corresponding to the G₂+M (M=mitosis) phase (4C level) or beyond were not detected, indicating the absence of dividing cells or of endoploidy (an increase in the number of chromosome sets caused by replication without cell division) in sugarcane leaves. Mean DNA content was significantly different ($P < 0.0001$) between the three groups of interspecific hybrids tested and among the genotypes within each of the groups (Table 2). Large differences (a 66 % variation) in DNA content (2C) were detected, ranging from 3.05 pg (*S. spontaneum* 'IND 81-146') to 8.91 pg (*S. officinarum* 'Muntok Java'), which corresponds to a genome size ranging from 1474 Mbp to 4298 Mbp per haploid nucleus (Table 3). Mean nuclear DNA content (7.15 pg/2C and 3451 Mbp/1C) for progeny from the PM cross was 1.14 and 1.25 times larger than those for progeny from the PCP (6.27 pg and 3026 Mbp) and from the GI crosses (5.67 pg and 2735 Mbp), respectively. Differences in DNA amount were 1.70, 1.20, and 1.23-fold within the GI, PCP, and PM F₁ progeny populations, respectively. The 2.92-fold difference obtained for the group of parents encompassed the variation observed in the three F₁ populations. The only difference noted within the commercial group was between CP 72-2086 and Louisiana Purple. This would

indicate that the genome size is stable in commercial cultivars even after approximately 12 generations (estimated via pedigree; J.D. Miller personal communication) of intercrossing advanced materials in the Canal Point breeding program.

Transmission

Overall, hybrids generally had mean DNA contents within the range of their parents. This corroborates the $n + n$ transmission observed by Burner (1997) and d'Hont et al. (1996) and contradicts the $2n + n$ transmission generally reported for interspecific hybrids between *S. officinarum* and *S. spontaneum* genotypes, particularly when *S. officinarum* is used as female. All of the PM progeny were within the range of values calculated for the two parents and all but one from the PCP cross. Six putative progeny with $2n + n$ transmission (five from the Green German x IND 81-146 cross and one from the Pin 84-1 x CP 70-1133 cross) were detected with mean DNA contents larger (in absolute values) than that of the larger-genome parent. However, three of the six clones were questionable and could be the result of self-pollination of either parent. Self-pollination is a common unintended byproduct of sugarcane crossing.

Correlations of DNA content with phenotype

DNA content was positively and significantly correlated ($r = 0.30$, $P < 0.001$) with plant height. Clones with a larger amount of DNA tended to be taller but to have lower and negative values for sugar yield components such as Brix ($r = -0.49$, $P < 0.0001$) and pol and sucrose content ($r = -0.41$, $P < 0.001$). Bennett (1976) suggested that variation in nuclear DNA content has a major impact on many plant traits. Burner (1997) suggested that $2n + n$ hybrids would be expected to have a significant impact in sugarcane yield because of the increase in chromosome number. The three putative $2n + n$ hybrid genotypes did not have the highest mean values for sugar yield components. Negative associations between DNA content and yield and growth parameters were also reported in another field study on corn (Biradar et al., 1994).

No association with stalk diameter and stalk weight was detected, contrary to Burner's (1997) expectations. Sink capacity in these interspecific F_1 progeny populations is limited since stalk diameter is usually two to three times smaller than that of the commercial cultivars, resulting in low but positive correlations between internode diameter and sugar juice analyses ($r = 0.43$, $P < 0.0001$ for Brix; $r = 0.42$, $P < 0.001$ for pol; and $r = 0.41$, $P < 0.001$ for sucrose content). Increasing stalk diameter to a commercial size requires backcrossing to commercial sugarcane cultivars or noble (*S. officinarum*) genotypes. In this study, genome size was not a good predictor of the phenotypic traits considered. However, bivariate density plots gave a better picture of the structure of the data, showing two distinct groups of interspecific hybrids when associating genome size with the phenotypic traits (Figure 2a, shown for Brix only). Further investigation revealed that the two groups were constituted by the GI group of F_1 hybrids at the lower end (Figure 2b) and by the combined PM-PCP groups at the higher end (Figure 2c) of the spectrum, with some clones overlapping between the two. In the GI group, most of the population with smaller DNA content had a higher mean Brix (Figure 2b) and in the PCP and PM groups, clones with larger DNA amount had a higher mean Brix (Figure 2c). However, the relationships may still be different when variation in DNA content is evaluated for commercial cultivars or different species.

Chromosome number and DNA content

The regression of chromosome number on genome size was highly significant ($P < 0.001$; $R^2 = 0.99$) (Figure 3). Knowing the 2C nuclear DNA content, chromosome numbers were predicted for the hybrid populations and other genotypes by the following equation: Chromosome number = $14.298 \times \text{DNA content (pg)} + 15.19$. Based on the prediction equation, chromosome numbers in the hybrid progeny ranged from $2n = 82$ to 128. These values are close to or within the range of values reported by Burner (1997) for some of these progeny. However, Burner did not identify the F_1 progeny used in his study.

DISCUSSION

Estimates showed that sugarcane has a relatively large and variable genome size (3.05-8.91 pg/2C), which is about 1.5 times that of corn and 10 times that of rice (*Oryza spp.*). These values are comparable to the DNA content estimates published by Arumuganathan and Earle (1991a). A larger variation in DNA content (3.05 to 5.31 pg) was observed between the two *S. spontaneum* species considered in this study. *S. spontaneum* cytotypes are considered to be the most variable of the *Saccharum* species (Lu et al., 1994). Genome size appeared to be relatively stable within *S. officinarum* species (6.32-6.66 pg) and within the CP commercial sugarcane cultivars (6.30-7.05 pg). It is premature to definitively state whether Green German and Muntok Java are in fact hybrids or true *S. officinarum* clones. Muntok Java, with its DNA content (8.91 pg) outside the range of values obtained for the *S. officinarum*, is more likely a hybrid with a considerable increase in genome size. The DNA content of Green German is comparable to that of the other *S. officinarum* species. However, a larger sample size would be needed to confirm these findings. A stable genome size can be explained by the fact that most sugarcane cultivars worldwide were derived from intercrossing the original progeny from a few parents or by indirect selection pressure. Similar stability of nuclear genome size was reported in other vegetatively propagated crops, like banana (*Musa spp.*) (Lysak et al., 1994). The polyploid nature of sugarcane buffers the crop from a rapid decrease in diversity and from a loss of heterozygosity. d'Hont et al. (1996) estimated the chromosomal contribution from *S. spontaneum* as 10 % in a modern commercial (R570) and an interspecific hybrid and observed a 10 % recombination between the two species.

The direction of the cross is important in the interspecific hybridization of sugarcane (Burner and Legendre, 1993; Burner, 1997; Sreenivasan et al., 1987). From a *S. officinarum* x *S. spontaneum* cross, more progeny with $2n + n$ chromosomes are expected, whereas, in the reverse or in a *S. spontaneum* x commercial cross, progeny with $n + n$ transmission are predominant. In this study, most of the F_1 hybrids appeared to have inherited DNA in the $n + n$ fashion, as their DNA content was within the range of DNA content of their respective parents but closer to that of the maternal parent. Large variations (16-40 %) were observed in the interspecific progeny of the three crosses. This might be attributed to amplification and deletions of chromosome segments leading to variations in the copy number of repeated sequences. Rayburn et al. (1993) suggested that variation in nuclear DNA content of maize F_1 hybrids was due to instability in DNA sequence copy number which depended on the parental combinations.

No progeny were found with a lower DNA content than that of the smaller genome parent. Four of the GI hybrids appeared to be putative $2n + n$ progeny, and one appeared to be

the product of self-pollination of the maternal parent. This confirmed d'Hont et al.'s (1996) finding that $n + n$ transmission is probably more common than previously reported in *S. officinarum* x *S. spontaneum* interspecific crosses.

The increase in DNA content in sugarcane did not necessarily affect phenotype in these interspecific populations. The association may, however, be population-dependent. Genome size was highly correlated with chromosome number, indicating that chromosome numbers can be derived from estimates of genome size. Correlation between genome size and ploidy level is common in plants (Palomino et al., 2003) and similar highly significant correlations were also found in buffalograss (*Buchloë dactyloides*) (Johnson et al., 1998) and fine fescues (*Festuca* spp.) (Huff and Palazzo, 1998). Further investigation with a greater number of sugarcane clones with known chromosome numbers would be necessary to confirm this finding. A quick and reliable determination of the ploidy level via flow cytometry would greatly alleviate tedious slide preparations for error-prone microscopic counting of chromosomes.

CONCLUSION

This study used flow cytometry to estimate DNA content and genome size in sugarcane interspecific populations and revealed that sugarcane has a large genome (3.05-8.91 pg). Analysis of the nuclear DNA content and genome size showed distinct differences among the sugarcane species considered, with some overlapping between the *S. officinarum* and commercial CP cultivars. Variation of the nuclear genome tended to be more extensive within the *S. spontaneum* species and among the F_1 progeny of a particular cross. Flow cytometry offers the potential, through determination of DNA content and genome size, to verify the true hybrid nature of progeny in interspecific crosses and consequently make genetic and genome analyses more efficient. The technique may help facilitate a better utilization of interspecific crosses aimed at transferring genes among different ploidy levels and at finding species or chromosome-specific markers for marker-assisted selection and breeding in sugarcane.

ACKNOWLEDGMENTS

The authors thank Dr. Randall Neidtz and assistants (USDA-ARS, Fort Pierce) for making the flow cytometer available and helping with the protocols, to Mr. Wesley Bronski for helping with sample collection and preparations, to interns, Miss Griselda Flores and Mr. Javaris Yarns, for helping throughout, and to Dr. Jaroslav Dolezel for providing the plant standards used in this study.

REFERENCES

1. Arumuganathan, K., and E.D. Earle. 1991a. Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.* 9:211-215.
2. Arumuganathan, K., and E.D. Earle. 1991b. Estimation of nuclear DNA contents of plants by flow cytometry. *Plant Mol. Biol. Rep.* 9:229-241.

3. Bennet, M.D. 1976. DNA content, latitude, and crop plant distribution. *Envir. Exp. Bot.*16:93-108.
4. Bennet, M.D., and I.J. Leitch. 1995. Nuclear DNA amounts in angiosperms. *Ann. Bot.* 76:113-176.
5. Bennet, M.D., and J.B. Smith. 1976. Nuclear DNA amounts in angiosperms. *Phil. Trans. Roy. Soc. London B*274:227-274.
6. Bennett, M.D., P. Bhandol, and I.J. Leitch. 2000. Nuclear DNA amounts in angiosperms and their modern uses: 807 new estimates. *Ann. Bot.* 86:859-909.
7. Berding, N., and B.T. Roach. 1987. Germplasm collection, maintenance, and use. Pages: 143-210. In: *Sugarcane Improvement Through Breeding*, D.J. Heinz, ed. Elsevier, New York.
8. Biradar, D.P., D.G. Bullock, and A.L. Rayburn. 1994. Nuclear DNA amount, growth, and yield parameters in maize. *Theor. Appl. Genet.* 88:557-560.
9. Bremer, G. 1961. Problems in the breeding and cytology of sugar cane. II. Sugar cane breeding from a cytological view-point. *Euphytica* 10:121-133.
10. Burner, D. 1997. Chromosome transmission and meiotic behavior in various sugarcane crosses. *J. Amer. Soc. Sugar Cane Technol.* 17:38-50.
11. Burner, D.M., and B.L. Legendre. 1993. Chromosome transmission and meiotic stability of sugarcane (*Saccharum spp.*) hybrid derivatives. *Crop Sci.* 33:600-606.
12. Burner, D.M., and B.L. Legendre. 1994. Cytogenetic and fertility characteristics of elite sugarcane clones. *Sugar Cane* 1994(1):6-10.
13. Cotter, F., S. Nasipuri, G. Lam, and B.D. Young. 1989. Gene mapping by enzymatic amplification from flow-sorted chromosomes. *Genomics* 5:470-474.
14. d'Hont, A., L. Grivet, P. Feldmann, S. Rao, N. Berding, and J.C. Glaszmann. 1996. Characterization of the double genome structure of modern sugarcane cultivars (*Saccharum spp.*) by molecular cytogenetics. *Mol. Gen. Genet.* 250:405-413.
15. d'Hont, A., D. Ison, K. Alix, C. Roux, and J.C. Glaszmann. 1998. Determination of basic chromosome numbers in the genus *Saccharum* by physical mapping of ribosomal RNA genes. *Genome* 41:221-225.
16. Daniels, J., and B.T. Roach. 1987. Taxonomy and evolution. Pages: 7-84. In: *Sugarcane Improvement Through Breeding*, D.J. Heinz, ed. Elsevier, New York.

17. De Laat, A.M.M., W. Godhe, and M.J. Vogelzang. 1987. Determination of ploidy of single plants and plant populations by flow cytometry. *Plant Breed.* 99:303-307.
18. Dolezel, J. 1997. Applications of flow cytometry for the study of plant genomes. *J. Appl. Genet.* 38:285–302.
19. Dolezel, J., P. Binarova, and S. Lucreti. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biol. Plant.* 31:113-120.
20. Dolezel, J., M. Dolezelova, and F.J. Novak. 1994. Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). *Biol. Plant.* 36:351-357.
21. Dolezel, J., S. Sgorbati, and S. Lucretti. 1992. Comparison of three fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiol. Plant.* 85:625-631.
22. Hammat, N., N.W. Blackwall, and M.R. Davey. 1991. Variation in the DNA content of *Glycine* species. *J. Exp. Bot.* 42:659-665.
23. Huff, D.R., and A.J. Palazzo. 1998. Fine fescue species determination by flow cytometry. *Crop Sci.* 38:445-450.
24. Johnson, P.G., T.P. Riordan, and K. Arumuganathan. 1998. Ploidy level determinations in buffalograss clones and populations. *Crop Sci.* 38:478-482.
25. Kenton, A.Y., P. Rudall, and A.R. Johnson. 1986. Genome size and variation in *Sisyrinchium* (Iridaceae) and its relationship to phenotype and habitat. *Bot. Gazette* 147:342-354.
26. Lu, Y.H., A. d'Hont, D.I.T. Walker, S. Rao, P. Feldmann, and J.C. Glaszmann. 1994. Relationships among ancestral species of sugarcane revealed by RFLP using single copy maize nuclear probes. *Euphytica* 78:7-18.
27. Lysak, M.A., M. Dolezelová, J.P. Horry, R. Swennen, and J. Dolezel. 1994. Flow cytometric analysis of nuclear DNA content in *Musa*. *Theor. Appl. Genet.* 98:1344-1350.
28. Lysak, M.A., and J. Dolezel. 1998. Estimation of nuclear DNA content in *Sesleria* (Poaceae). *Caryologia* 51:123-132.
29. Nair, M.K. 1975. Cytogenetics of *Saccharum officinarum* L. and *S. spontaneum* L. IV. Chromosome number and meiosis in *S. officinarum* x *S. spontaneum* hybrids. *Caryologia* 28:1-14.

30. Palomino, G., J. Dolezel, I. Mendez, and A. Rubluo. 2003. Nuclear genome size analysis of *Agave tequiliana* Weber. *Caryologia* 56:37-46.
31. Price, S. 1960. Cytological studies in *Saccharum* and allied genera. VI. Chromosome numbers in *S. officinarum* and other noble sugar canes. *Hawaii Plant. Rec.* 56:183-194.
32. Rayburn, A.L., D.P. Biradar, D.G. Bullock, and L.M. McMurphy. 1993. Nuclear DNA content in F₁ hybrids of maize. *Heredity* 70:294-300.
33. Roach, B.T. 1969. Cytological studies in *Saccharum*. Chromosome transmission in interspecific and intergeneric crosses. *Proc. Int. Soc. Sugar Cane Technol.* 13:901-920.
34. SAS Institute Inc. 2002. SAS/STAT 9.0 User's Guide. SAS Institute Inc., Cary, North Carolina.
35. Sreenivasan, T., B.S. Ahloowalia, and D.J. Heinz. 1987. Cytogenetics. Pages: 211-253. In: *Sugarcane Improvement Through Breeding*, D.J. Heinz, ed. Elsevier, New York.
36. Steel, R.G.D., and J.H. Torrie. 1980. *Principles and Procedures of Statistics*. McGraw-Hill, New York.
37. Van Dilla, M.A., and L.L. Deaven. 1990. Construction of gene libraries for each human chromosome. *Cytometry* 11:208-218.

Table 1. Crosses and their F₁ hybrid derivatives, *S. officinarum* clones, and sugarcane cultivars used for determination of DNA content and genome size.

Genotypes	Number	Crosses and types†
F ₁ hybrids	25	Green German x IND 81-146
F ₁ hybrids	26	Pin 84-1 x Muntok Java
F ₁ hybrids	7	Pin 84-1 x CP 70-1133
Black Cheribon		<i>S. officinarum</i>
Louisiana Purple		<i>S. officinarum</i>
Oi Dang		<i>S. officinarum</i>
CP 72-1210		Commercial cultivar
CP 72-2086		Commercial cultivar
CP 80-1827		Commercial cultivar
CP 81-1254		Commercial cultivar
CP 89-2143		Commercial cultivar
NCo310		Commercial cultivar

† Green German = *S. officinarum* sp., IND 81-146 and Pin 84-1 = *S. spontaneum* sp.

Table 2. Analysis of variance for DNA content measured on a set of sugarcane genotypes (F₁ hybrids and their parents, cultivars, and *S. officinarum* clones).

Source	df	Mean Square
Model	72	1.66**
Group	4	12.45**
Genotypes(Group)	68	1.04**
Residual†	62	0.07

** significant at $P < 0.01$.

† Residual could be written as Plant(Genotype Group).

Table 3. DNA content and genome size for three groups of sugarcane interspecific hybrids and their parents, commercial cultivars, and wild relatives (*S. officinarum*).

Genotypes	Family†	Chromosome‡	DNA content	Genome size	Tukey's grouping§
		no.	pg	Mbp	
M. Java	P	138-144‡	8.91 ± 0.13	4297.8	A
CP 70-1133	P	114‡	6.78 ± 0.15	3269.1	B
G. German	P	102-117‡	6.71 ± 0.29	3238.8	B
PIN 84-1	P	96‡	5.31 ± 0.04	2562.4	C
IND 81-0146	P	56‡	3.05 ± 0.03	1473.7	D
US 99-1021	PCP	114	6.89 ± 0.46	3326.2	A
US 99-1023	PCP	108	6.45 ± 0.26	3112.2	A
US 99-1024	PCP	103	6.13 ± 0.20	2954.9	A
US 99-1026	PCP	103	6.12 ± 0.21	2952.6	A
US 99-1027	PCP	103	6.12 ± 0.23	2952.6	A
US 99-1025	PCP	103	6.09 ± 0.00	2939.5	B
US 99-1022	PCP	96	5.62 ± 0.22	2710.4	B
US 99-1015	PM	127	7.79 ± 0.10	3757.8	A
US 99-1013	PM	124	7.60 ± 0.14	3667.2	AB
US 99-1054	PM	123	7.52 ± 0.60	3625.8	ABC
US 99-1050	PM	123	7.51 ± 0.18	3623.1	ABC
US 99-1018	PM	123	7.50 ± 0.05	3616.6	ABC
US 99-1043	PM	122	7.47 ± 0.02	3603.7	ABC
US 99-1019	PM	121	7.40 ± 0.05	3568.3	ABC
US 99-1012	PM	120	7.33 ± 0.10	3537.4	ABCD
US 99-1047	PM	120	7.32 ± 0.15	3531.7	ABCD
US 99-1057	PM	120	7.27 ± 0.06	3504.8	ABCD
US 99-1052	PM	119	7.24 ± 0.13	3490.2	ABCD
US 99-1016	PM	119	7.21 ± 0.11	3479.7	ABCD
US 99-1051	PM	119	7.21 ± 0.06	3478.7	ABCD
US 99-1044	PM	119	7.20 ± 0.14	3475.3	ABCD
US 99-1056	PM	118	7.14 ± 0.24	3444.8	ABCD
US 99-1045	PM	117	7.06 ± 0.06	3407.1	ABCD
US 99-1053	PM	117	7.06 ± 0.12	3407.6	ABCD
US 99-1055	PM	116	7.03 ± 0.14	3391.2	ABCD
US 99-1059	PM	115	6.98 ± 0.42	3363.6	ABCD
US 99-1058	PM	115	6.94 ± 0.08	3346.8	BCD
US 99-1046	PM	114	6.91 ± 0.42	3334.2	BCD
US 99-1048	PM	114	6.91 ± 0.05	3329.9	BCD
US 99-1020	PM	114	6.90 ± 0.46	3328.8	BCD
US 99-1017	PM	112	6.75 ± 0.02	3257.0	CD
US 99-1049	PM	109	6.55 ± 0.05	3159.8	D
US 99-1014	PM	109	6.51 ± 0.10	3140.5	D

Table 3. Cont'd. DNA content and genome size for three groups of sugarcane interspecific hybrids and their parents, commercial cultivars, and wild relatives (*S. officinarum*).

Genotypes	Family†	Chromosome‡	DNA content	Genome size	Tukey's groupings§
		no.	pg	Mbp	
US 99-1033	GI	129	7.90 ± 0.05	3812.2	A
US 99-1002	GI	120	7.33 ± 0.00	3536.7	AB
US 99-1030	GI	116	7.04 ± 0.33	3392.6	AB
US 99-1040	GI	116	7.01 ± 0.00	3380.3	AB
US 99-1041	GI	114	6.86 ± 0.24	3312.6	B
US 99-1009	GI	110	6.62 ± 0.37	3192.5	B
US 99-1031	GI	92	5.35 ± 0.13	2579.3	C
US 99-1011	GI	91	5.30 ± 0.05	2556.4	C
US 99-1029	GI	91	5.25 ± 0.01	2533.4	C
US 99-1003	GI	90	5.23 ± 0.04	2522.8	C
US 99-1008	GI	90	5.22 ± 0.02	2517.7	C
US 99-1001	GI	89	5.15 ± 0.09	2484.6	C
US 99-1037	GI	89	5.11 ± 0.03	2466.0	C
US 99-1004	GI	88	5.09 ± 0.12	2456.2	C
US 99-1006	GI	88	5.07 ± 0.05	2446.4	C
US 99-1032	GI	88	5.06 ± 0.03	2443.5	C
US 99-1042	GI	88	5.06 ± 0.00	2441.7	C
US 99-1038	GI	87	5.01 ± 0.01	2417.3	C
US 99-1039	GI	86	4.92 ± 0.02	2375.5	C
US 99-1005	GI	86	4.91 ± 0.05	2368.6	C
US 99-1010	GI	86	4.89 ± 0.01	2361.3	C
US 99-1034	GI	85	4.88 ± 0.03	2354.9	C
US 99-1035	GI	84	4.78 ± 0.16	2303.2	C
US 99-1007	GI	84	4.76 ± 0.03	2296.1	C
US 99-1036	GI	83	4.72 ± 0.10	2275.7	C
CP 72-1210	C	116	7.05 ± 0.29	3398.8	A
CP 72-2086	C	116	7.01 ± 0.01	3378.9	AB
NCo310	C	112‡	6.86 ± 0.23	3311.1	AB
CP 89-2143	C	112	6.74 ± 0.28	3248.8	AB
CP 80-1827	C	112	6.73 ± 0.15	3245.1	AB
Black Cheribon	C	80‡	6.66 ± 0.09	3211.4	AB
Oi Dang	C	80‡	6.62 ± 0.24	3194.8	AB
CP 80-1743	C	109	6.55 ± 0.06	3160.0	AB
CP 81-1254	C	106	6.33 ± 0.39	3053.6	B
La Purple	C	80‡	6.32 ± 0.14	3046.7	B

† Grouping of the genotypes as C for commercial, P for parents, and the others as progeny of the following crosses: Green German x IND 81-146 (GI), Pin 84-1 x Muntok Java (PM), and Pin 84-1 x CP 70-1133 (PCP).

‡ Chromosome numbers as estimated by Burner (1997), others as predicted from genome size.

§ By group-ANOVA with different letters within a group indicating significance at $P < 0.05$.

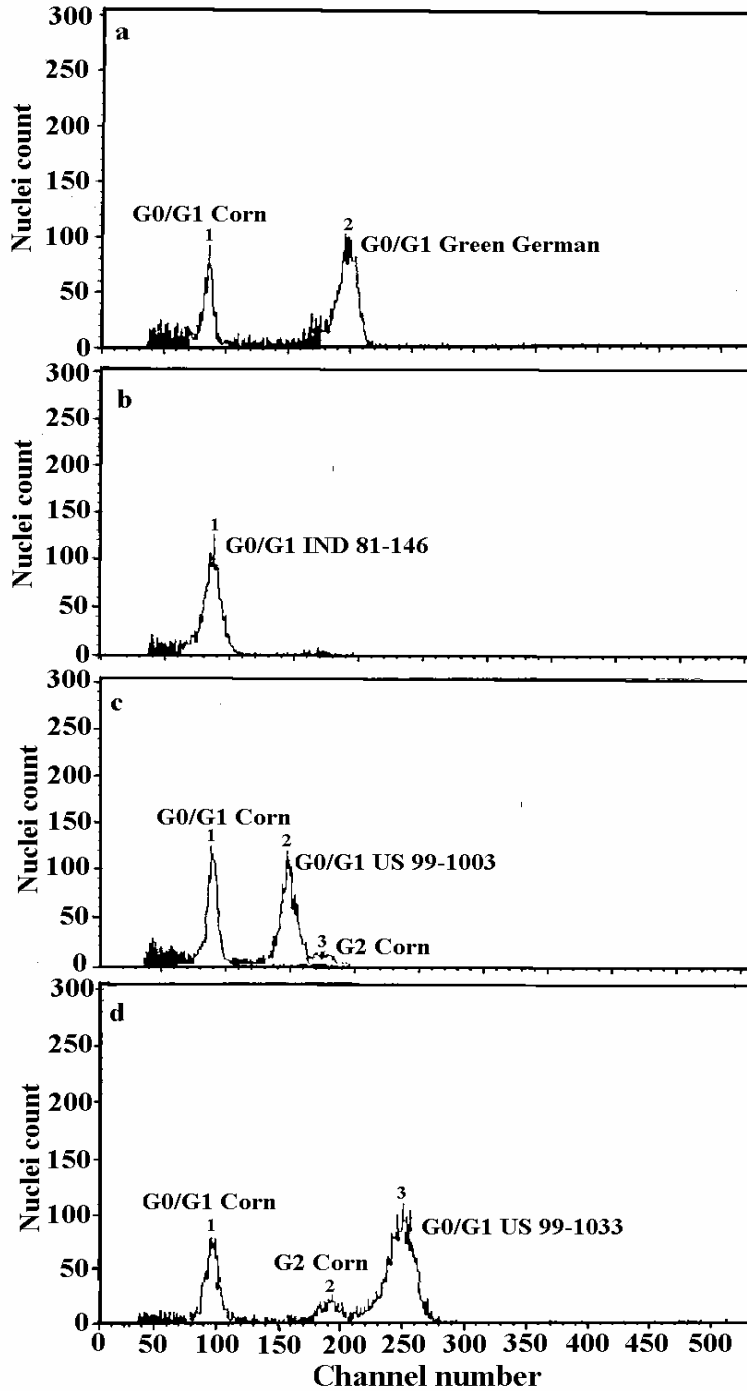


Figure 1. Histograms of relative fluorescence intensities obtained after cytometry analyses of DAPI-stained nuclei of sugarcane and corn young leaf tissues. Corn ($2C=5.43$ pg) was used as the internal standard. a) Green German, b) IND 81-146 alone as its G_0/G_1 peak coincided with that of corn when co-run, c) US99-1003, a $n+n$ progeny of the Green German x IND 81-146 cross with an intermediate DNA content, and d) US 99-1033, a putative $2n+n$ progeny of the same cross, as its peak was further located than that of the larger-genome parent. The G_0/G_1 and G_2 peaks correspond to the first ($2C$ DNA content) and second ($4C$ DNA content) periods of cell growth of the cell cycle, respectively.

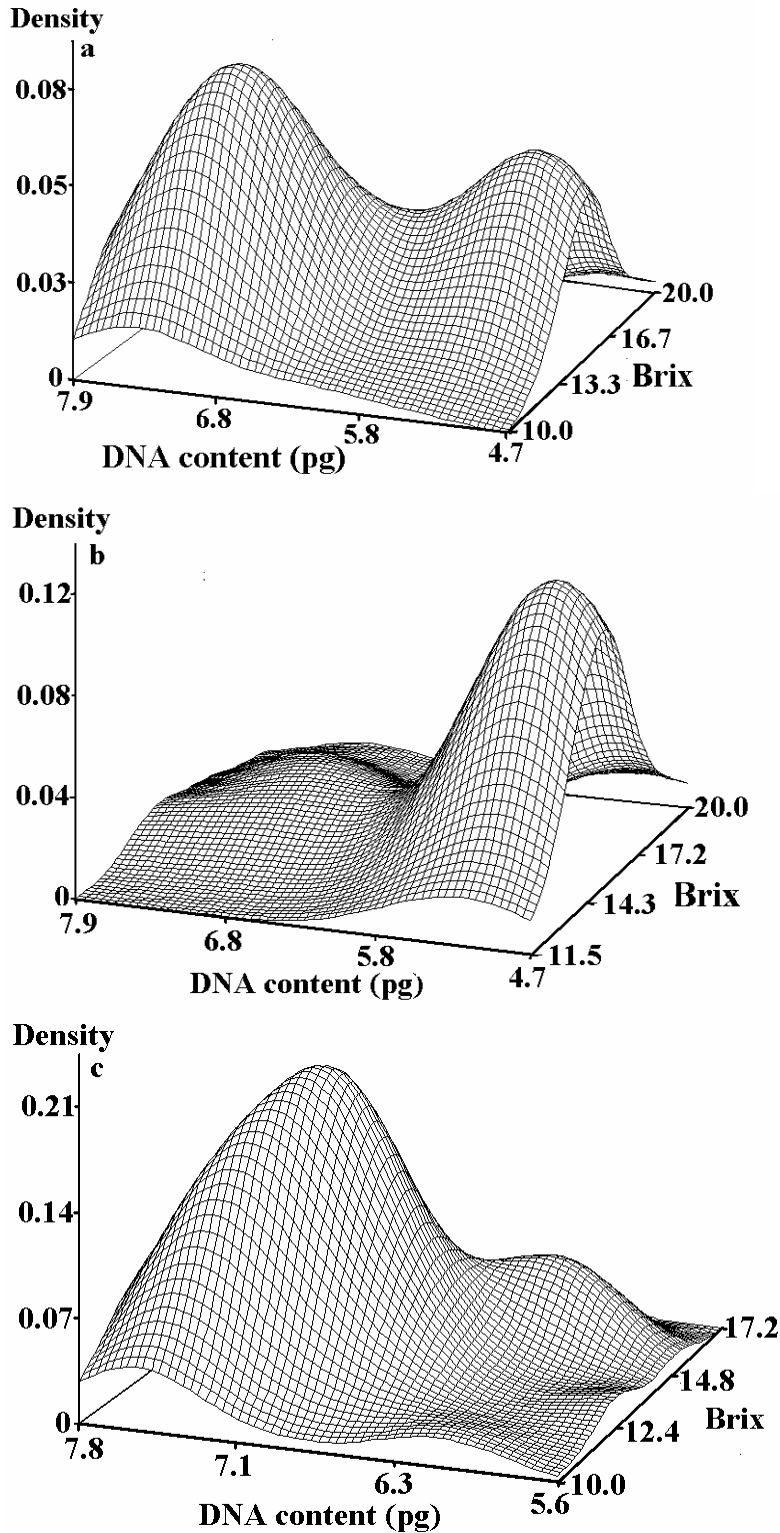


Figure 2. Bivariate density plots relating DNA content with Brix in a) all three populations b) F_1 progeny of Green German x IND 81-146, and c) F_1 progeny of Pin 84-1 x Muntok Java and Pin 84-1 x CP70-1133 crosses.

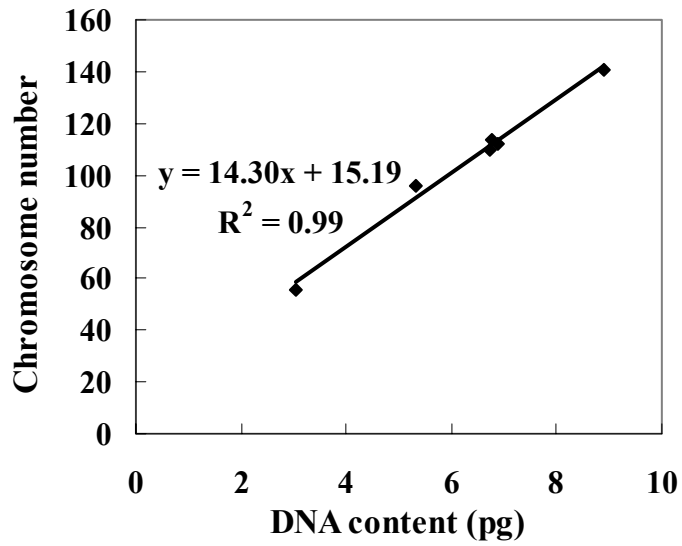


Figure 3. Relationship between genome size and chromosome numbers in a set of *Saccharum* spp. (y = chromosome number and x = 2C DNA content in pg; $P < 0.000$)