Differential detection of transposable elements between *Saccharum* species

Marislane Carvalho Paz de Souza, Jéssica Naiana Silva and Cicero Almeida

Laboratório de Recursos Genéticos, Universidade Federal de Alagoas, Campus de Arapiraca, Arapiraca, AL, Brazil.

Abstract

Cultivars of sugarcane (*Saccharum*) are hybrids between species *S. officinarum* (*x = 10, 2n = 8x = 80*) and *S. spontaneum* (*x = 8, 2n = 5-16x = 40 - 128*). These accessions have 100 to 130 chromosomes, 80-85% of which are derived from *S. officinarum*, 10-15% from *S. spontaneum*, and 5-10% are possible recombinants between the two genomes. The aim of this study was to analyze the repetition of DNA sequences in *S. officinarum* and *S. spontaneum*. For this purpose, genomic DNA from *S. officinarum* was digested with restriction enzymes and the fragments cloned. Sixty-eight fragments, approximately 500 bp, were cloned, sequenced and had their identity analyzed by DNA hybridization on membrane, using genomic probes from *S. officinarum* and *S. spontaneum*. The hybridization experiment revealed that six TEs had a similar repetitive DNA pattern in the genomes of *S. officinarum* and *S. spontaneum*. The hybridization experiment revealed that six TEs had a similar repetitive DNA pattern in the genomes of *S. officinarum* and *S. spontaneum*. We concluded that the species *S. officinarum* and *S. spontaneum* have differential accumulation LTR retrotransposon families, suggesting distinct insertion or modification patterns.

Keywords: sugarcane, dot-blot hybridization, LTR retrotransposons, repetitive DNA.

Received: December 10, 2012; Accepted: May 3, 2013.

Sugarcane (*Saccharum spp.*) is a cultivated plant of significant economic importance, as it accounts for 70% of all sugar production in the world. In recent years, due to the global energy crisis, it has also emerged as an excellent source of renewable energy by the production of ethanol. This cultivated plant belongs to the genus *Saccharum*, family Poaceae, and the main species are *S. officinarum*, *S. spontaneum*, *S. robustum*, *S. sinense*, *S. barberi* and *S. edule*. Modern varieties of sugarcane are a complex of polyploids and aneuploids (Grivet and Arruda, 2001), originating from the recombination of different hybrids derived from two highly polyploid species, *S. officinarum* (*x = 10, 2n = 8x = 80*) and *S. spontaneum* (*x = 8, 2n = 5-16x = 40 - 128*). Usually, they have between 100 and 130 chromosomes, 70-80% of which are derived from *S. officinarum*, 10-20% from *S. spontaneum* and approximately 10% are recombinants between the two species (D’Hont et al., 1996, Piperidis et al., 2010). Furthermore, it is treated as an extremely large genome, with small chromosomes, which complicates the understanding of their genetic architecture and taxonomy (Pan et al., 2000).

The *Saccharum* genus presents taxonomic difficulties due to the existence of cross-hybridization producing “synthetic species” and to polymorphisms due to ploidy and aneuploidy, besides high selective pressure caused by genetic improvement. Relationships between the genera *Saccharum*, *Erianthus*, *Sclerostachya* and *Narenga* have suggested that they constitute an interrelated group involved in the origin of sugarcane, being called the “Saccharum complex” (Mukherjee, 1957). In addition to these genera, *Miscanthus* Anderss. section *Diantra* Keng, *Erianthus* Mickx. section *Ripidium* Henrard, and *Sclerostachya* (Hack) were included in the Saccharum complex (Daniels and Roach, 1987). Other phylogenetic analyses performed in *Miscanthus*, *Saccharum* and other close genera showed that the many species of *Saccharum* are closer to the *Miscanthus* species than to other species of those genera (Hodkinson et al., 2002). However, molecular data have shown only two true species in the genus *Saccharum*, called *S. spontaneum* and *S. officinarum*, which include the wild *S. robustum* and the races *S. barberi*, *S. sinense* and *S. edule* (Irvine, 1999; Grivet et al., 2004).

There are several strategies for analyzing these relationships, one of which is the differential amplification of repetitive DNA sequences, widely studied in several organisms (Ugarkovic and Plohl, 2002). Thus, determining the distribution of repetitive DNA sequences in species of ge-
nus *Saccharum* and close genera will permit a better understanding of the relationships between the genomes of these species and of the taxonomy of the group, besides the sequencing of the genome. The transposable elements (TEs) have been reported to be responsible for improving the genome. They show great diversity, with different families in plants, and with differences among individuals of the same species (reviewed by Morgante et al., 2007). In sugarcane, there are reports on TEs such as the one of Domingues et al. (2012) who described 35 families within four *Copia* and *Gypsy* lineages, and the one of Kajihara et al. (2012), showing that TEs in sugarcane are transcriptionally active, however, to our best knowledge, no analysis based on DNA hybridization showing differences between species of genus *Saccharum* has been published so far. In this work, we aimed to evaluate the abundance of transposable elements in *S. officinarum* and *S. spontaneum*.

Buds of *S. officinarum* and *S. spontaneum* accessions were obtained from the Serra do Ouro germplasm bank and germinated in pots. Young leaves were collected from each accession, genomic DNA was extracted using the CTAB method, as described by Saghai-Marof et al. (1984), and quantified in 1% agarose gel. 10 μg of DNA from *S. officinarum* (cultivar Lousier) was digested using the restriction enzyme *MboI* (Fermentas), following its protocol. The digestion products were separated in a 2% agarose gel, and a region of 200 to 500 bp of the gel was excised and DNA purified. The fragments were then cloned into the vector CloneJET (Fermentas) and transformed in *E. coli*. The clones were confirmed by PCR, using 50 μL of reaction solution containing 50 ng of DNA, 1x enzyme buffer, 1.5 mM of MgCl₂, 0.2 mM of dNTPs, 0.5 U of *Taq*-polymerase (Fermentas) and 0.2 μM of each primer. The DNA was then submitted to 30 amplification cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. A DNA standard of 1 kb molecular weight was used for the determination of the molecular weight of the respective fragments of amplified DNA. The amplification products were separated in a 1% agarose gel and visualized using ethidium bromide.

The clones were amplified by PCR, the products were visualized on 1% agarose gels and sent to Macrogen (South Korea) for purification and DNA sequencing using primers from vector. The sequences were analyzed by BLAST against GenBank sequences and the genomes of rice, maize and sorghum. The max target sequences (100), which automatically adjust parameters for short input sequences, and the expected number of chance matches in a random model (expected threshold = 10) were used as BLAST general parameter.

DNA from clones containing partial TEs was placed (dots) onto a nylon membrane (Hybond N+, Amersham Biosciences) and fixed at 120 °C for 30 min. The membrane was pre-hybridized in a solution of DIG Easy Hyb (DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science) for 30 min. Probes were produced from genomic DNA of *S. officinarum* and *S. spontaneum* digested with *MboI* and labeled with digoxigenin-AP by “Random Primer” following the manufacturer’s instructions (DIG High Prime DNA Labeling and Detection Starter Kit II). The probes were denatured at 100 °C for 5 min, added to the hybridization solution at 37 °C, and the mix was then placed onto the membrane and hybridized for 12 hours at 37 °C. After hybridization, the membrane was washed for 2x 5 min in 2x SSC and 0.1% SDS, followed by two washes of 15 min in 0.1x SSC and 0.1% SDS. Hybridization was visualized in a reaction with CSPD (Disodium 3-(4-methoxyspiro [1,2-dioxetane-3,2’-[5’-(2-chloro)tricyclo [3.3.1.1²,7]decan]-4-yl]phenylphosphate) (Roche Applied Science) and the signals evidenced by exposure to X-ray film. DNA hybridization was performed with three replicates, and the experimental controls were: single copy clone (JX101456 - partial tubulin-specific chaperone E), repetitive clone (Scent7 - described as repetitive by Nagaki et al., 1998), and the genomic DNA controls.

Genomic DNA of the *S. officinarum* accessions (Louzier and IJ76-530) was digested for 20 hours with the enzyme *EcoRI* (Fermentas), after which the fragments were separated in a 2% agarose gel at 30 V for 6 h. Then the gel was washed in depurination, denaturation and neutralization solutions. After 24 h, the DNA was fixed on a nylon membrane (Hybond N+, Amersham Biosciences) at 80 °C for 2 h. The membrane was pre-hybridized in a solution of DIG Easy Hyb (DIG High Prime DNA Labeling and Detection Starter Kit II) for 30 min. The probes were labeled with a random primer using PCR clones Soff.a4, Soff.e4 and Soff.f2 DNA and then denatured at 100 °C for 5 min and added to the hybridization solution at 37 °C for 12 hours. After hybridization, the membrane was washed for 2x 5 min in 2x SSC and 0.1% SDS, followed by two 15 min washes in 0.1x SSC and 0.1% SDS. The hybridization was visualized in a reaction with CSPD and the signals evidenced by exposure to X-ray film.

The sequences were analyzed using the NCBI sequence database for maize mobile elements, in the genomes of rice, maize and sorghum. Twelve clones showed analogies with TEs deposited in the databases, suggesting that they are abundant in the genome of *S. officinarum* (Table 1). Clone Soff.a4, which is highly repeated in the genome of *S. officinarum* and *S. spontaneum*, has a similarity with a centromeric sequence belonging to the SCEN family (Table 1), described by Nagaki et al. (1998). The same clone had also high similarity to the LTR retrotransposon Maximus family, reported in sugarcane by Domingues et al. (2012). For the grass species, BLAST showed 11 repetitive clones in sorghum, seven in maize and four in rice (Table 1).

Two membranes composed of 14 clones (12 clones containing partial TEs and two controls) and two genomes were hybridized with genomic probes from *S. officinarum*.
and S. spontaneum, respectively. A stringency of 80% was
used, to allow high specificity of the sequences with the
genomes analyzed. The results showed clear dots when
compared with the genomic controls (genomic DNA from
S. officinarum and S. spontaneum), the DNA repetitive con-
trol and the single copy control (Figure 1).

Some clones showed greater signal intensity in the
genome of S. officinarum (Soff.a2, Soff.b7, Soff.c3, Soff.e4, Soff.e6 and Soff.f2) (Figure 1A), whereas other
clones had similar signal intensity in the two genomes (Soff.a4, Soff.a9, Soff.b11, Soff.b12, Soff.d8 and Soff.d11)
(Figure 1B). A clone of repetitive DNA (Scent7) for the ge-
nus Saccharum, described by Nagaki et al. (1998), was
added as a repetition control, and the signal showed similar-
ity to Soff.a9 and Soff.d11, indicating consistency in the
hybridizations and confirming that the sequences are repet-
itive (Figure 1C).

The Soff.a4, Soff.e4 and Soff.f2 (JX101454, JX101448 and JX101442 sequences, respectively) were
analyzed by Southern blot in two S. officinarum accessions,
in order to obtain their repetition patterns in the genome, us-
ing the same conditions as for dot blot hybridization. The
results showed that these sequences were dispersed in the
genome of S. officinarum, appearing as a smear in the two
accessions analyzed (Figure 2).

Cultivated sugarcane accessions are formed by two
main genomes which correspond to the pure species S.
officinarum and S. spontaneum. A process of backcrossing
associated with “nobilization” allowed to combine a frac-
tion of 80-85% from S. officinarum with 10-15% from S.
spontaneum. Both species are polyploid, S. officinarum
with 2n = 80 and S. spontaneum with 2n = 40-128 (DHont
et al., 1998), and recent phylogenetic studies have shown
that the genus Saccharum is monophyletic, comprising
only two true species (S. officinarum and S. spontaneum).
Their speciation is relatively recent, as it dates back to ap-
proximately 1.5-2 million years (Jannoo et al., 2007), sug-
gesting similar DNA sequences. In the present study, we

Table 1 - BLAST results for repetitive DNA sequences of Saccharum officinarum

| Gene bank N. | Clone name | Repetitive in: sorghum/maize/rice | Order | Superfamily | Family | e-value |
|-------------|------------|-----------------------------------|-------|-------------|--------|---------|
| JX101444    | Soff.a2    | y/n/n                             | LTR   | Copia       | Maximus/Sire | 3e-125  |
| JX101454    | Soff.a4    | *y/n/n                            | LTR   | Copia       | Maximus/Sire | 1e-08   |
| JX101446    | Soff.a9    | y/n/n                             | LTR   | Copia       | Maximus/Sire | 2e-37   |
| JX101450    | Soff.b7    | y/y/y                             | LTR   | CopiaGypsy  | Maximus/SireDEL/TEKAY | 5e-48  |
| JX101445    | Soff.b11   | n/n/y                             | LTR   | Copia       | Maximus/Sire | 8e-66   |
| JX101451    | Soff.b12   | **y/y/n                           | LTR   | Copia       | Maximus/Sire | 9e-125  |
| JX101443    | Soff.c3    | n/y/n                             | LTR   | Gypsy       | DEL/TEKAY   | 4e-59   |
| JX101452    | Soff.d8    | y/y/n                             | LTR   | Copia       | Maximus/Sire | 5e-28   |
| JX101453    | Soff.d11   | y/y/n                             | LTR   | Copia       | Maximus/Sire | 1e-63   |
| JX101448    | Soff.e4    | y/y/y                             | LTR   | Copia       | Maximus/Sire | 8e-180  |
| JX101455    | Soff.e6    | y/n/n                             | LTR   | Copia       | Maximus/Sire | 1e-48   |
| JX101442    | Soff.f2    | y/y/y                             | LTR   | Gypsy       | DEL/TEKAY   | 1e-77   |
| JX101456    | Soff.a10   | n/n/n                             | -     | -           | -       | -      |

*Only chromosome #3; **only chromosomes #3, 5, 6 and 7.

Figure 1 - Hybridization profile for Saccharum officinarum (1) and S. spontaneum (2) genomic probes. (A) clones with greater intensity in the
genome of S. officinarum; (B) highly repetitive clones in the two genomes;
(C) repetition control (Scent7) and clone as a single copy (Soff.a10); and
(D) positive control with probe from genomic DNA of S. officinarum
(S.off) and S. spontaneum (S. spo).
amplification of repetitive DNA was observed by Naka-
sequences are common to several species, and differential al.

Species

Saccharum officinarum

Alix K, Pualet F, Glaszmann JC and D’Hont A (1998) Isolation and characterization of a satellite DNA family in the Saccharum complex. Genome 41:854-864.

Alix K, Pualet F, Glaszmann JC and D’Hont A (1999) Inter-Alu-like species-specific sequences in the Saccharum complex. Theor Appl Genet 99:962-968.

Araujo PG, Rossi M, de Jesus EM, Saccaro NL, Kajihara D, Massa R, de Felix JM, Drummond RD, Falco MC, Chabregas SM, et al. (2005) Transcriptionally active transposable elements in recent hybrid sugarcane. Plant J 44:707-717.

Bowen NJ and Jordan K (2002) Transposable elements and the evolution of eukaryotic complexity. Curr Issues Mol Biol 4:65-76.

D’Hont A, Grivet L, Feldmann P, Rao S, Berding N and Glaszmann JC (1996) Characterisation of the double genome.

used repetitive DNA sequences to identify differential abundance of the TEs in the genomes of S. officinarum and S. spontaneum, using DNA hybridization. As both species are polyploid, differential accumulation of repetitive DNA sequences may occur since speciation. Indeed, differentially amplified sequences were detected indirectly by D’Hont et al. (1996) and Piperidis et al. (2010), using GISH to identify the genomes.

The fraction of dispersed repetitive DNA is the major component in many eukaryotic genomes, being the largest contributor to variation in DNA content between similar organisms (Zhao et al., 1998). Mobile DNA elements have contributed significantly to this increase, by being selfish DNA and by multiplying in a disorderly way in the genomes (Bowen and Jordan, 2002). TEs described by Domingues et al. (2012) in the sugarcane genome from the R570 (BAC hybrid) are transcriptionally active (Araujo et al., 2005), and a search of repetitive DNA has identified species-specific repeated DNA in Saccharum (Kim et al., 2011). However, there is no information about differential accumulation of TEs between the Saccharum species. In the present study, the repetitive DNA sequences were similar to TEs, suggesting that these elements are highly abundant in the genomes of the Saccharum species and must have contributed to the expansion of the genomes. Additionally, six of the DNA sequences analyzed were more abundant in the genome of S. officinarum, suggesting differential accumulation between genomes.

Studies of repetitive DNA in Saccharum have shown a large number of repetitive sequences in this group (Alix et al., 1998, 1999; Nagaki and Murata, 2005) in which some sequences are common to several species, and differential amplification of repetitive DNA was observed by Nakayama (2004), showing accumulation of a specific sequence in the genome of S. officinarum and suggesting that repetitive DNA amplification is a common evolutionary mechanism in the genomes. Indeed, collinearity between sugar-

References

Alix K, Baurens FC, Paulet F, Glaszmann JC and D’Hont A (1998) Isolation and characterization of a satellite DNA family in the Saccharum complex. Genome 41:854-864.

Acknowledgments

This work received support from the Breeding Program for Sugarcane at the Federal University of Alagoas and from the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, Brazil).

Figure 2 - Southern blot of Saccharum officinarum, accession Lousier (1) and I76-530 (2). Genomic DNA was digested with EcoRI (A) and hybridized with the probes Soff.c4 (JX101448) (B), Soff.a4 (JX101454) (C), and Soff.f2 (JX101442) (D).
structure of modern cultivars (*Saccharum* spp.) by molecular cytogenetics. Mol Gene Genet 250:405-413.

Daniels J and Roach BT (1987) Taxonomy and evolution. In: Heinz DJ (ed) Sugarcane Improvement Though Breeding. Elsevier, Amsterdam, pp 7-84.

Domingues D, Cruz GMQ, Nogueira FTS, Vicentini R, Alves C de S, Van Sluys MA (2012) Analysis of plant LTR-retrotransposons at the finescale family level reveals individual molecular patterns. BMC Genomics 13:e137.

Garsmeur O, Charron C, Bocs S, Jouffe V, Samain S, Couloux A, Droc G, Zini C, Glaszmann JC, Van Sluys MA, *et al.* (2011) High homologous gene conservation despite extreme autopolyploid redundancy in sugarcane. New Phytol 189:629-642.

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

Grivet L, Daniels C, Glaszmann JC and D’Hont A (2004) A review of recent molecular genetics evidence for sugarcane evolution and domestication. Ethnobot Res Appl 2:9-17.

Hodkinson TR, Chase MW, Lledó MD, Salamin N and Renvoiza SA (2002) Phylogenetics of *Miscanthus, Saccharum* and related genera (*Saccharinae, Andropogoneae, Poaceae*) based on DNA sequences from ITS nuclear ribosomal DNA and plastid *trnL* intro and *trnL-F* intergenic spacers. J Plant Res 115:381-392.

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

Jannoo N, Grivet L, Chantret N, Garsmeur O, Glaszmann JC, Arruda P and D’Hont A (2007) Orthologous comparison in a gene-rich region among grasses reveals stability in the sugarcane polyploidy genome. Plant J 50:574-585.

Kajihara D, de Gogoy F, Hamaji TA, Blanco SR, Van Sluys MA and Rossi M (2012) Functional characterization of sugarcane mustard domesticated transposases and comparative diversity in sugarcane, rice, maize and sorghum. Genet Mol Biol 35:632-639.

Kim C, Robertson JS and Paterson A (2011) Inference of sub-genomic origin of BACs in an interspecific hybrid sugarcane cultivar by overlapping oligonucleotide hybridizations. Genome 54:727-737.

Morgante M, Paoli E and Radovic S (2007) Transposable elements and the plant pan-genomes. Curr Opin Plant Biol 10:149-155.

Murkherjee SK (1957) Origin and distribution of *Saccharum*. Bot Gaz 119:55-61.

Nagaki K and Murata M (2005) Characterization of CENH3 and centromere-associated DNA sequences in sugarcane. Chromosome Res 13:195-203.

Nagaki K, Tsujimoto H and Sasakuma T (1998) A novel repetitive sequence of sugar cane, SCEN family, locating on centromeric regions. Chromosome Res 6:295-302.

Nakayama S (2004) Species-specific accumulation of interspersed sequences in genus *Saccharum*. Genes Genet Syst 79:361-365.

Pan YB, Burner DM and Lendendrei BL (2000) An assessment of the phylogenetic relationship among sugarcane and related taxa based on the nucleotide sequence of 5S rRNA intergenic spacers. Genetica 108:285-295.

Pedrosa-Harand A, Almeida CCS, Mosiolek M, Blair MW, Schweizer D and Guerra M (2006) Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris* L.) evolution. Theor Appl Genet 112:924-933.

Piperidis G, Piperidis N and D’Hont A (2010) Molecular cyto-genetic investigation of chromosome composition and transmission in sugarcane. Mol Genet Genomics 284:65-73.

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population-dynamics. Proc Natl Acad Sci USA 81:8014-8018.

Ugarkovic D and Plohl M (2002) Variation in satellite DNA profiles - Causes and effects. EMBO J 21:5955-5959.

Wang, J, Roe B, Macmil S, Yu Q, Murray JE, Tang H, Chen C, Najar F, Wiley G, Bower J, *et al.* (2010) Microcollinearity between autopolyploid sugarcane and diploid sorghum genomes. BMC Genomics 11:e261.

Zhang P, Li W, Friebe B and Gill BS (2006) Simultaneous painting of three genomes in hexaploid wheat by BAC-FISH. Genome 47:979-987.

Zhao X, Si Y, Hanson RE, Crane CF, Prince HJ, Stelly DM, Wendel JF and Paterson AH (1998) Dispersed repetitive DNA has spread to new genomes since polyploid formation in cotton. Genome Res 8:479-492.

**Internet Resources**

Rice genome http://rice.genomics.org.cn/rice (accessed September 22, 2012).

Maize genome http://www.maizegdb.org (accessed September 22, 2012).

Sorghum genome http://www.phytozome.net (accessed September 22, 2012).

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.