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Short Communication

Differential detection of transposable elements between *Saccharum* species

Marislane Carvalho Paz de Souza, Jéssica Naiana Silva and Cícero 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 in NCBI, and in the rice, maize, and sorghum genome databases using BLAST. Twelve clones containing partial transposable elements, one single-copy control, one DNA repetitive clone control and two genome controls were 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*, while six TEs were more abundant in the genome of *S. officinarum*. 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.

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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).

Send correspondence to Cícero Almeida. Laboratório de Recursos Genéticos, Universidade Federal de Alagoas, Campus de Arapiraca, Avenida Manoel Severino Barbosa s/n, Rodovia AL 115, km 6,5, 57309-005 Arapiraca, AL, Brazil. E-mail: cicerocarlos@pesquisador.cnpq.br.

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-Maroof *et al.* (1984), and quantified in 1% agarose gel. 10 μg of DNA from *S. officinarum* (cultivar Lousier) was digested using the restriction enzyme *Mbo*I (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 $MgCl_2$, 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 *Mob*I 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'-chloro)tricyclo [3.3.1.1^{3,7}]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 (Lousier and IJ76-530) was digested for 20 hours with the enzyme *Eco*RI (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 Soffa.4, 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*

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.

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 control 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 genus *Saccharum*, described by Nagaki *et al.* (1998), was added as a repetition control, and the signal showed similarity to Soff.a9 and Soff.d11, indicating consistency in the hybridizations and confirming that the sequences are repetitive (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, using 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 fraction 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 approximately 1.5-2 million years (Jannoo *et al.*, 2007), suggesting similar DNA sequences. In the present study, we

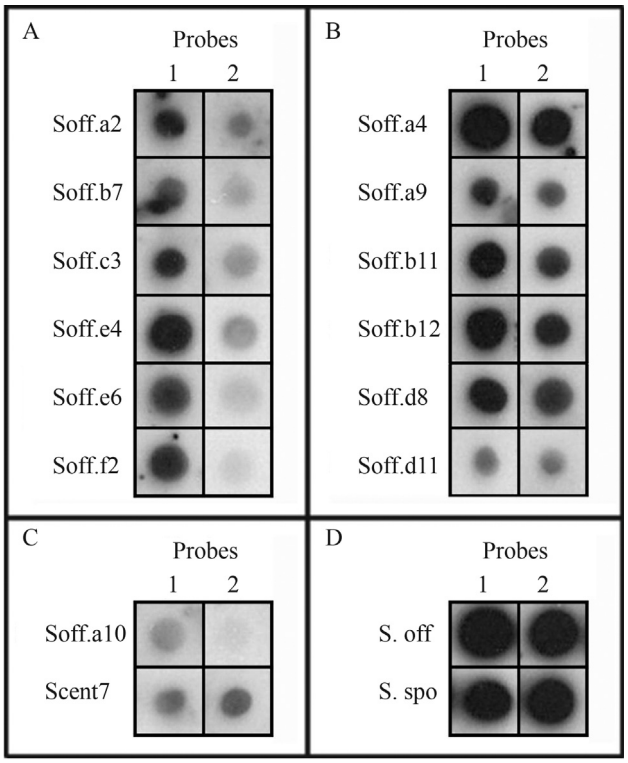

Figure 1 - Hybridization profile for *Saccharum officinarum* (1) and *S. spontaneum* (2) genomic probes. (A) clones with greater intensity in the genome of *S. officinarum*; (B) highly repetitive clones in the two genomes; (C) repetition control (Scent7) and clone as a single copy (Soff.a10); and (D) positive control with probe from genomic DNA of *S. officinarum* (S.off) and *S. spontaneum* (S. spo).

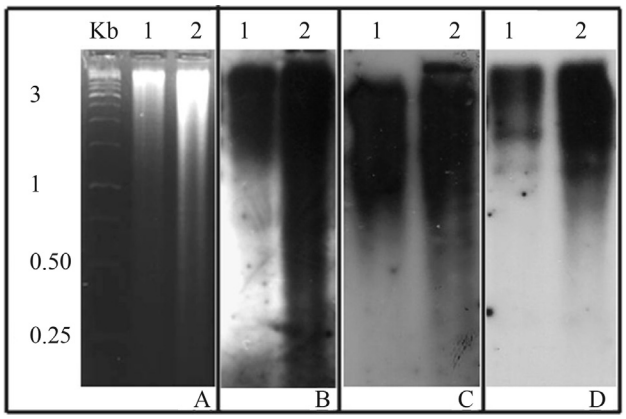

Figure 2 - Southern blot of *Saccharum officinarum,* accession Lousier (1) and IJ76-530 (2). Genomic DNA was digested with *Eco*RI (A) and hybridized with the probes Soff.e4 (JX101448) (B), Soff.a4 (JX101454) (C), and Soff.f2 (JX101442) (D).

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 sugarcane and sorghum was described by Wang *et al.* (2010) for 20 genomic regions, and unaligned regions between sugarcane and sorghum sequences are occupied mostly by repeats. Garsmeur *et al.* (2011) showed that sugarcane and sorghum genomes are collinear in the genic regions and present high structure and sequence conservation. These results suggest that the main mechanism of divergence among species is the repetitive component of their genomes. In other species, as well as in the genus *Phaseolus*, the differences between species are probably due to differential amplification of the repetitive DNA (Pedrosa-Harand *et al.*, 2006). Furthermore, Zhang *et al.* (2006) showed species-specific accumulation repetitive DNA in *Triticum*.

Repetitive sequences of the SCEN family were found to occupy 0.6% of the sugarcane genome, or 2.6 x 10^5 copies per haploid genome (Nagaki *et al*., 1998). Our results showed that SCEN sequences are distributed within the genome of *S. officinarum* and S. *spontaneum* with a high number of repetitions, confirming the findings of Nagaki *et al*. (1998). The SCENT7 sequence (SCEN family) was used as repetitive control DNA. Its signal was less than that of SCENM9 (similar to Soff.a4), however the SCEN family showed a large divergence in the sequence, suggesting that members of this family were differentially amplified in the genomes of *S. officinarum* and *S. spontaneum.* We conclude that the species *S. officinarum* and *S. spontaneum* have differential repetitive DNA, belonging to LTR retrotransposons families, suggesting a distinct pattern of insertion or modification, which is the important factor for increasing the differences between genomes in the genus *Saccharum*.

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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).

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