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Molecular Characterization of Cultivated Sugarcane Varieties on Tabasco, Mexico.

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Abstract

In recent years different types of morphological, biochemical and molecular methods have been used to characterize plant varieties. The aim of this study was to characterize molecular genetic variability in 12 cultivated sugarcane (*Saccharum* spp.) varieties in the state of Tabasco Mexico by AFLP. Young leaves of plants growing in the field were selected for experiment. After testing 12 combinations of primers, the combination E-ACC/M-CTA produced from 72 to 1353 bp polymorphic fragments. The dendrogram revealed two distinct groups of varieties and a variety different from both groups. The varieties C 87-51, ATM 96-40, B 4362, Mex 69-290, Mex 57-1285 and Mex 91-130 integrated the Group I. Such varieties formed a cluster with a similarity of 0.77% among them. The varieties RD 75-11, Mex 79-431, SP 70-1284, Mex 59-32 and CP 72-2086 integrated the Group II. Such varieties formed another cluster with a similarity of 0.70% among them. The variety Mex 68-P-23 was different from the other 11 varieties.

Key words: *DNA; AFLP; Molecular markers*

Introduction

The increase in world sugar production is due to the introduction of improved cultivars (Jackson, 2005). The modern cultivars of sugar cane (*Saccharum* spp) currently planted in the world resulted from the first interspecific cross among S. officinarum, S. spontaneum and S. barberi carried out at the beginning of the last century (Grivet and Arruda, 2002). Several authors as Baksha et al. (2002) and Lu *et al.* (1994) have pointed out the need to create new sugarcane germplasm with a higher sugar yield, high resistance to abiotic and to biotic factors and with easy agronomic management.

Saccharum officinarum is an octoploid species with a chromosomal number 2n = 70 - 140 (Irvine, 1999). Therefore, and due to phenomena of euploidy and aneuploidy, commercial clones could be the product of numerous hybridizations with high chromosome numbers 2n = 100 - 130 (Grivet and Arruda, 2002). Thus, in the generation of new sugarcane varieties the characterization of varieties is an important aspect.

Recently, progress has been made in searching for genes of interest to characterize plant varieties by using various types of markers. The biochemists and DNA-based markers are the most widely used. Some of them are: Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Microsatellites or Simple Repeated Sequences (SSR). These markers allow the identification and isolation of genes of interest through DNA amplification by the Polymerase Chain Reaction (PCR) technique. They are currently being used in the genetic tracing of sugarcane (Hoarau et al., 2001; Rossi et al., 2003), as well as in deducing inferences about genetic variability and interrelationships between genotypes at the level of DNA (Lima et al., 2002; Cordeiro et al., 2003), among which the construction of genetic maps in commercial varieties stands out (Aitken et al., 2005).

The AFLP marker combines RFLP and PCR technique and consists of the amplification of multiple arbitrary regions of the genome (Vos et al., 1995). The use of AFLP has been successful to get distributed molecular markers in eukaryotic genomes and in cultivated plants that have a low rate of DNA polymorphism, as well as in the detection and evaluation of genetic variation in germplasm collections and biodiversity studies (Vuylsteke et al., 2000). Therefore, AFLP marker was chosen to carry out the molecular characterization in varieties of sugar cane from the state of Tabasco.

Materials and Methods

Sugarcane varieties

Twelve sugarcane varieties from the germplasm bank located in the experimental field of the Postgraduate College - Campus Tabasco were used (Table 1).

Variety	Parents	Country of origin
C 87-51	Co 281 x POJ 2878	Cuba
Mex 57-1285	CP 52-43 x CB 45-6	Mexico
Mex 59-32	B 35-187 x CP 34-120	Mexico
Mex 91-130	Mex 57-280 x Mex 72-161	Mexico
ATM 96-40	SP 70-6180 x Mex 79-431	Mexico
RD 75-11	CB 38-22 x CP 57-603	República Dominicana
Mex 79-431	Co 421 x Mex 57-473	Mexico
B 4362	B 37-161 x POJ 2878	Barbados
Mex 69-290	Mex 56-476 x Mex 53-142	Mexico
SP 70-1284	CB 41-76 x	Brasil
CP 72-2086	CP 62-374 x CP 63-588	Estados Unidos
Mex 68-P-23	Mex 59-84 x	Mexico

Table 1: *Cultivated sugarcane varieties (Saccharum spp.) in the state of Tabasco Mexico, used to determine its genetic variability.*

Sugarcane plants growing on field were used to sample young leaves. Samples were collected according to the protocol of the applied molecular genetics laboratory of the International Maize and Wheat Improvement Center (CIMMYT, 2006).

DNA extraction

Baindrige et al. (1990). DNA concentration for each sample was determined by the spectrometric method. Then the concentrations of all samples were equalized to 100 ng μl^{-1}. The AFLPs procedure was performed following the recommendations indicated in the AFLP®Analysis System I Kit and AFLP® Starter Primer Kit (InvitrogenTM, Carlsbad, CA).

Genomic DNA digestion

Genomic DNA was digested using restriction enzymes EcoRI and MseI. A mixture of 5X Buffer reaction (5 µl), tomato control DNA (100 ng in 5 µl (2.5 µl)), DNA sample (250 ng in 18 µl), EcoRI/MseI (2 µl), distilled water (15.5 µl) for a final volume of 25 µl. It was capped at 37°C during 2h.

Ligation of adapters

For ligation, 24 µl of ligation solution adapters and 1 µl of T4 DNA ligase were used to prepare a 1:5 dilution.

Pre-amplification

The pre-amplification was performed with a 1:10 dilution of the Primer Mix (40 µl), 10X PCR buffer plus Mg (5.0 µl), Taq DNA polymerase (1 µl). The resulting solution was taken to a DNA thermocycler (Bio-Rad Engine Peltier Thermal Cycler), previously programmed for 20 cycles at 94°C during 30s, 56°C during 1 min and 72°C during 1 min. 1:10 dilutions with water were prepared for AFLP (Sigma) from the pre- amplification.

Selective amplification

According to the manufacturer's recommendations, possible combinations of primers were sought to find the ones that provide the best banding pattern and thus be able to differentiate the 12 varieties of sugarcane (Table 2).

Data analysis

The bands, from the electrophoretic patterns obtained from the molecular tests, were evaluated in a binary way (0 absence, 1 presence). The coefficient of genetic similarity between each pair of genotype was calculated from the matrices of the original data by using only the polymorphic bands. The NTSys PC 2.0 statistical package (dendrogram and genetic distance matrix) was used.

Combination Number	Primer combination Eco RI / Mse I
1	E-AAC/M-CAC
2	E-AAG/M-CTC
3	E-ACA/M-CTT
4	E-ACC/M-CTA
5	E-AAC/M-CAG
6	E-AAC/M-CTG
7	E-ACC/M-CTG
8	E-ACG/M-CAC
9	E-ACG/M-CAA
10	E-ACT/M-CTC
11	E-AGC/M-CAT
12	E-AGG/M-CAT

Table 2: *Primer combinations used for the molecular characterization of 12 cultivated sugarcane varieties (Saccharum spp.) in the state of Tabasco, Mexico.*

Results and Discussion

The amplification products evaluated to visualize and carry out analysis of polymorphisms with AFLP markers allow us to observe genetic differences among the analyzed varieties. After performing 12 combinations of these amplifiers, the E-ACC/M-CTA combination showed the highest polymorphism among varieties. The size of the polymorphic fragments detected with this primer combination ranged from 72 to 1353 bp (Figure 1).

The low polymorphism detected indicates the narrow genetic base of the 12 evaluated varieties cultivated in the Tabasco state Mexico. This narrow genetic base may be due to the high polyploidy and the frequent aneuploidy of the genus Saccharum (Grivet and Arruda, 2002). Moreorver sugarcane genome is complex ranging from 2500-4000 million base pairs (Mbp/1C) (Arumuganathan and Earle, 1991). Only the Saccharum offcinarum chloroplast DNA genome is 141182 bp (Asano et al., 2004; Calsa et al., 2004).

Another reason for the low polymorphism observed may be that the crosses between genotypes focus mainly on the generation of varieties with resistance to pests and diseases (Harvey et al., 1994). These crosses have been successful and have originated varieties of sugarcane more productive but with a marked reduction in its genetic base (Jannoo et al., 1999).

The AFLP technique has been successful in sugarcane in several countries. In Brazil, Lima et al. (2002) detected an average of 50% polymorphism among 79 cultivars. In India, Selvi et al. (2006) reported an average of 52% polymorphism among 28 cultivars by using 12 primer combinations.

In Mexico, Rodríguez et al. (2005) characterized the 15 most sowed sugarcane varieties by using 12 AFLP combinations. 55.3% of their 884 generated markers were polymorphic, the Mex 73-523 y Mex 68-P-23 varieties were in the dendrogram further branches and were apart to the other varieties.

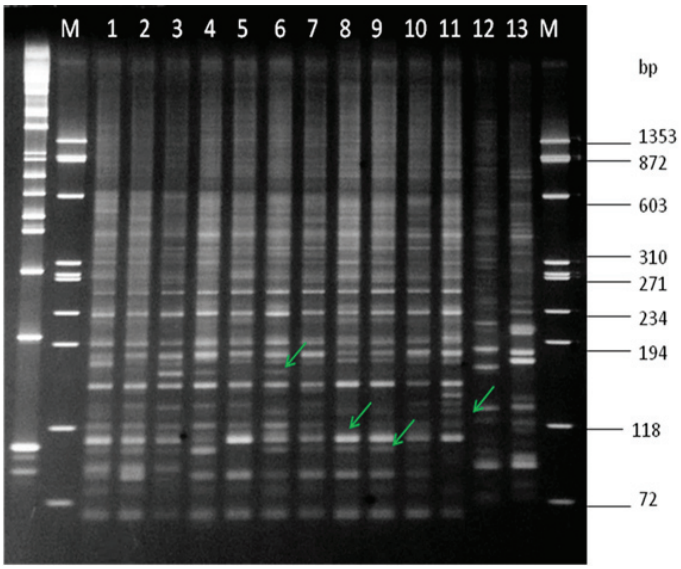

Figure 1: *E-ACC/M-CTA combination. Varieties: 1) C 87-51; 2) Mex 57-1285; 3) Mex 59-32; 4) Mex 91-130; 5) ATM 96-40; 6) RD 75-11; 7) Mex 79-431; 8) B 4362; 9) Mex 69-290; 10) SP 70-1284; 11) CP 72-2086; 12) Mex 68-P-23; 13) control +; M) Φ X174 / HáeIII marker. (Arrows indicate dispersion of polymorphic bands).*

Raboin et al. (2008) studied 72 Saccharum spp clones using the AFLP technique. They found 1537 polymorphic markers with 42 combinations. Among these markers, only 463 were located on the genetic map.

The dendrogram revealed three different groups of Saccharum spp. and a variety different from both groups (Figure 2). The varieties C 87-51, ATM 96-40, B 4362, Mex 69-290, Mex 57-1285 and Mex 91-130 integrated the Group I. Such varieties presented a 0.77% genetic similarity, i.e. these materials showed more genetic closeness among them compared to the varieties of group II. Group II included the varieties RD 75-1, Mex 79-431, SP 70-1284, Mex 59-32 and CP 72-2086. These varieties formed a conglomerate with 70% genetic similarity among them.

The Mex 68-P-23 variety was the one with the least genetic similarity (22%) compared to the rest of the analyzed varieties.

Rodríguez et al. (2005) reported that the 15 sugarcane varieties most used in the production in Mexico formed a narrow genetic population and that the Mex 68-P-23 variety had a greater genetic distance. The latter could indicate an increase in genetic variability, which is essential for genetic improvement. These and our results agree to the multiple studies citing out the narrow genetic base of modern cultivars (Grivet et al., 1996; Canales et al., 2003).

The sugarcane genotypes study has shown that there is a small degree of DNA diversity among modern varieties (Arro, 2005). In Cuba, Arencibia et al. (2006) found that the current commercial varieties of sugarcane present low genetic variability among them.

The above and our results can be explained by the "nobilization process". This process mean that the crosses of the modern cultivars used as parents of the first crosses were backcrossed several times with S. officinarum. As result of "nobilization" the new hybrids showed a reduction in their genetic base (Deren, 1995; Jannoo et al., 1999).

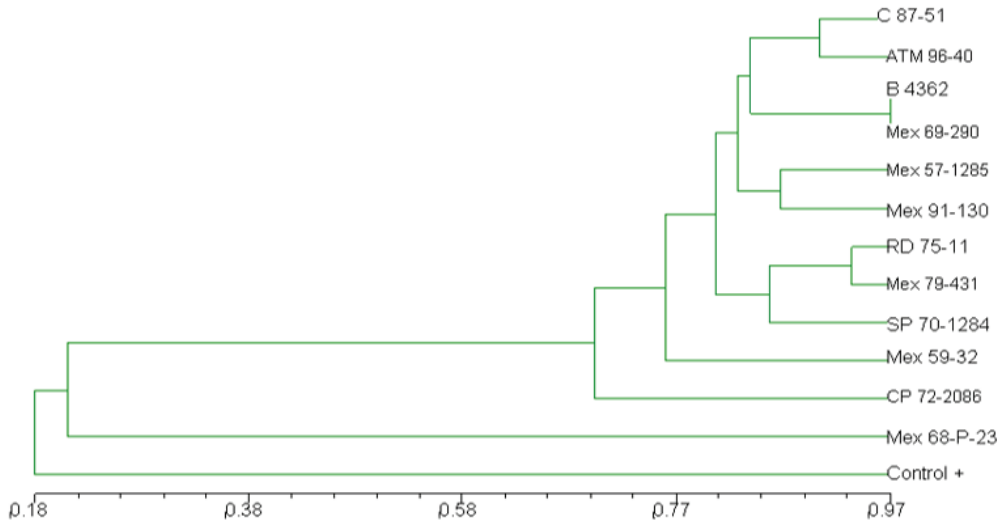

Figure 2: *Cluster analysis based on the genetic similarity AFLP of 12 cultivated sugarcane varieties in the state of Tabasco Mexico by the NTSYS PC program.*

Conclusions

The results confirm the usefulness of AFLP to obtain the necessary number of markers for genome analysis, as well as to characterize and to detect polymorphisms of 12 sugarcane varieties in the state of Tabasco.

The E-ACC / M-CTA combination was the one that produced the largest polymorphic fragments. A small degree of diversity at the DNA level among the cultivated sugarcane varieties in the state of Tabasco Mexico was found. The Mex 68-P-23 variety presented a faraway genetic distance with respect to the rest of the analyzed varieties.

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