Use of differential levels of mean observed heterozygosity in microsatellite loci of commercial varieties of sugarcane (*Saccharum* spp)

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ABSTRACT. In this study, we measured the genetic diversity within and among a set of 9 commercial sugarcane varieties used for alcohol and sugar production using 17 microsatellite DNA markers. The UGSM148 and UGSM59 primers were monomorphic for all 74 sugarcane samples. The estimated proportion of simple sequence repeated (SSR) polymorphic loci was 88.23%; 17 alleles were detected. The mean gene diversity of all SSR loci was 0.7279. The

highest observed heterozygosity (H_o) value was found in the RB72454 variety, whereas the lowest H_o value was recorded in the SP813250 variety. The SP813250, RB845210, and RB835054 sugarcane varieties were the most genetically uniform varieties. An extremely high level of population differentiation was detected in the varieties exhibiting similar agronomic characteristics. Analysis of the genetic structure of the 9 sugarcane varieties using SSR markers was especially important to identify SSR loci with high levels of heterozygosity and to identify varieties showing the highest levels of heterozygosity. The monomorphic primers may be used to evaluate the genetic stability of sugarcane during cycles of vegetative multiplication, i.e., propagation via rhizomes.

Key words: Sugarcane; Simple sequence repeat locus; Microsatellite; Genetic diversity; Genetic stability

INTRODUCTION

Recent use of sugarcane (*Saccharum* spp) as a source of ethanol and sugar has helped drive the economy of Brazil. In 2010/2011, 624 million tons of sugarcane was produced and subsequently used in the production of 33 million tons of sugar and 27.6 billion liters of ethanol fuel (<http://jornalcana.com.br/>). The Brazilian sugar and alcohol production system consists of 437 units of production, including 168 alcohol factories, 16 sugar factories, and 253 combined sugar and alcohol factories. Moreover, the bagasse from each ton of sugarcane processed could be used to generate electric energy or to produce additional ethanol. Because bagasse and sugarcane stalk straw have a similar cellulose:lignin:hemicellulose composition, the latter fulfills the same energy-providing purpose (Zanin et al., 2000). Cellobiase and endoglucanase from different sources and different types of pre-treatment are used in the processing of sugarcane bagasse (Martín et al., 2002; Neureiter et al., 2002; Hernández-Salas et al., 2009). Sugarcane bagasse is also used to produce cellulose film (Ruzene et al., 2009) and as an additive material in the production of cement (Sousa, 2009). Additionally, the sugarcane plant is used in the production of biodiesel in the form of sugarcane-derived ethanol, which is a component that is used during the processing of biodiesel in Brazil. Molasses is used for fermentation, and the vinasse is used as a field fertilizer. Sugarcane plants are therefore a symbol of renewable energy for the country (Lee and Bressan, 2006).

The broad utility of sugarcane has stimulated an interest in increasing the production and the development of new varieties that are adapted to different and heterogeneous regions of the country using breeding programs. Sugarcane improvement has been considered an important investment for the development of new varieties. However, Brazilian sugarcane germplasms have low genetic diversity and a narrow genetic base (Barbosa et al., 2000; Almeida et al., 2005). A narrow genetic base is undesirable for sugarcane breeding because a certain level of parental divergence is needed to create productive hybrids. Knowing the genetic diversity of the commercial sugarcane varieties and how they are genetically structured is important for both sugarcane breeding programs and the industrial sector that processes the by-products of sugarcane. High genetic diversity is important for the breeding programs, while genetically uniform varieties are of interest for the industrial sectors.

The cultivated sugarcane varieties were produced by interspecific hybridization between *Saccharum officinarum*, *S. robustum*, *S. barberi*, and the 2 wild species *S. spontaneum* and *S. sinense*. Because of its diverse origin, sugarcane is thought to have one of the most complex plant genomes, carrying variable chromosome numbers. Modern cultivars generally have between 100 and 130 chromosomes (Grivet and Arruda, 2002). *In situ* hybridization studies have suggested that modern cultivars inherit approximately 80% of their chromosomes entirely from *S. officinarum*, approximately 10% entirely from *S. spontaneum*, and approximately 10% as the result of recombination among the remaining ancestral species (D'Hont et al., 1996).

The objectives of this study were to measure the genetic diversity within and among commercial sugarcane varieties used for alcohol and sugar production through polymorphic microsatellite DNA markers. The marker systems that are used in studies of sugarcane include microsatellites or simple sequence repeats (SSR) (Cordeiro et al., 2000, 2001; Cordeiro and Henry, 2001; Pan et al., 2003; Pinto et al., 2004). Considerable advances have been made in the area of sugarcane genetics through the use of microsatellite markers. The associations of markers with sugarcane diseases (Wei et al., 2006) and sugar contents (Singh et al., 2008, 2011) have been investigated using sugarcane clones. Microsatellite markers have many desirable genetic attributes, including hypervariability, wide genomic distribution, co-dominant inheritance, reproducibility, multi-allelic nature, and chromosome-specific location. Knowing the observed heterozygosity (H_o) levels, as well as the differences among the sugarcane varieties, would help us to identify the genotypes that have the highest genetic diversity and measure the genetic divergence between varieties showing similar agronomic characteristics.

MATERIAL AND METHODS

Sugarcane samples

A set of 9 sugarcane varieties was chosen to represent part of the germplasm broadly cultivated in the northern region of Parana State and available to the alcohol and sugar factories (Fazenda Junqueira, Alto Alegre district in Colorado, PR, Brazil). The set includes sugarcane varieties from breeding programs at Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético and Centro de Tecnologia Canavieira. The selections included the following varieties: RB72454, RB845210, RB935744, RB867515, RB835054, RB855453, RB92579, SP813250, and RB036152. The morphological and agronomic characteristics of the varieties are shown in Table 1. The main characteristics of these 9 cultivars that are important for alcohol and sugar production are high productivity and sucrose content, wide adaptability, and resistance to diseases.

Microsatellite selection

DNA was isolated from young leaves (removed from the cartridge) of 8 plants of each of the RB845210, RB935744, RB867515, RB835054, RB855453, RB92579, SP813250, and RB036152 varieties and 10 plants of the RB72454 variety (74 samples in total) according to the procedure used by Hoisington et al. (1994). The 40 SSR primers previously mapped for sugarcane by the International Sugarcane Microsatellite Consortium (Cordeiro et al., 2000; Singh et al., 2008; Oliveira et al., 2009) were synthesized by Invitrogen Technologies Corporation (USA) and used with 5 DNA samples from each sugarcane variety. After screening

the 40 SSR primers, a set of 17 SSR primers (Table 2) was selected for further analysis. Polymerase chain reaction (PCR) was performed using a Techne TC-512 thermal cycler. The amplifications were performed by the Touchdown (Td) PCR program (Don et al., 1991) using a primer-specific annealing temperature (TA).

Table 1. Agronomic characteristics and origin of the sugarcane varieties from the Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético (RB varieties) and Centro de Tecnologia Canavieira (SP variety) breeding programs.

Variety	Agronomic characteristics	Parents
RB72454	High productivity; wide adaptability	CP53-76 X ?
RB845210	High productivity; great sucrose content	RB72454 X SP70-1143
RB935744	High productivity; excellent health	RB835089 X RB765418
RB867515	High productivity; high sucrose content	RB72454 X ?
RB036152	Precocity	SP835073 X RB867515
RB835054	High productivity; wide adaptability	RB72454 X NA56-79
RB855453	High productivity; high sucrose content	TUC71-7 X ?
RB92579	High productivity; resistant to pests	RB75126 X RB72199
SP813250	High productivity; high sucrose content	CP70-1547 X SP71-127

Table 2. Simple sequence repeat (SSR) primers previously mapped for sugarcane and the specific annealing temperature (TA) for each primer used in polymerase chain reaction (PCR) of the sugarcane varieties.

Primer	SSR (5'→3')	Size (bp)	TA	Forward (F) and reverse (R) primer sequence
SMC1237FL	(CA) ₆	100-140	58	F: TTCACGAACACCCACCTA R: GCGCCAGGTAACCTACTGAA
SMC1047HA	(GA) ₂₆	110-170	62	F: TGAGCCTAAGCCAGAAAGAAG R: GGAACCTAATTTCTACGAGAACAC
SMC2017FL	(CTT) ₁₃	210-260	62	F: CACAAGTGAAGATAATAGTGTCCCT R: GATCCCAAATCCCTTGATCTC
SMC16SA	(CA) ₁₁	90-130	58	F: GCAACCGGTCCTCATAC R: GAGCTTCTGATCGGCCTTC
SMC226CG	(CA) ₁₀	136	TD	F: GAGGCTCAGAAGCTGGCAT R: ACCCTCTATTTCCGAGTTGGT
UGSM148	(GAA) ₅ GGGCA...(GAG) ₅	42-647	TD	F: GATGACTCCTTGTTGGTGG R: CTTGACGACCTGCTGCT
UGSM59	(GGC) ₁₇	71-500	55	F: GTGAACGACTCCATCGCC R: TTGAAACCACCGTAAATAAGA
UGSM38	(ACG) ₇	50-1185	55	F: CCGAGTGATGATGTGATGT R: GGGACAACCTAATGTAAGTATT
UGSM117	(GCT) ₅ GCCAT...(TG)	93-867	TD	F: GAGGAGGTGACGGTGGAG R: GACGCTACGGTTCATTCA
SEGMS240	(GTT) ₁₂	100-900	53	F: CAACAAGAAAGAACACAATGAG R: AATGTGAATAACTAGGTGGTCC
SEGMS1069	(AGA) ₁₀	100-600	53	F: CGGGAATTCGATTTTCATGGGTTT R: GACTACTCTAAGCATCGTCTCTC
ESTB130	(CAA) ₁₃	124	50	F: GCCCAGGTAATATCCAGACTC R: GCTGTTGCTCACTGGTTCC
ESTB41	(CGA) ₈	163	63	F: CATGGAGAGCTGGGCGACCTG R: GCGCGCGGCGAGGATGA
ESTB145	(CCT) ₅	234	68	F: GGGAAAGCAAGCGAGAGCAGCAGAG R: GAGCGCGAGGCCGTTGTTGAG
ESTB60	(TTG) ₁₀	198	53	F: AGCCGCAATGAATCCAACTG R: CTCTAGCTCCGACGATGATACCTC
ESTC66	(CCGC) ₃	152	61	F: AGTACAGGCTGCTCTCAATCAA R: TCTGTCTCTGTGTTCTGTTCTG
mSSCIR56	(GT) ₂₄	125-170	58	F: ATTTGACGCTACGATGGTG R: ATCCGTTTTTCAGCAGAGC

Cordeiro et al. (2000); Singh et al. (2008); Oliveira et al. (2009). TD = Touchdown (Don et al., 1991).

For Td-PCR, 20- μ L volumes containing 25 ng genomic DNA; 10 mM Tris-HCl, pH 8.8; 2.0 μ L 10X reaction buffer (20 mM Tris-HCl, pH 8.0, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1.0 mM dithiothreitol, and 50% (v/v) glycerol); 2.5 mM $MgCl_2$; 0.1 mM each dATP, dGTP, dCTP, and dTTP; 0.3 μ L each primer (F and R primers); and 1 unit Taq polymerase (Invitrogen) were used. The PCR conditions were as follows: initial denaturation at 94°C for 1 min; 10 cycles of 1 min at 94°C, 1 min with an initial temperature of 65°C and a reduction of 1°C per cycle, and 2 min at 72°C; and 20 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. The final extension was 5 min at 72°C. The PCR using specific temperatures for the primer annealing was 94°C for 5 min followed by 30 cycles of 94°C for 1 min and the TA of each primer for 1 min, and later cycles with 72°C for 1 min. The final extension was 15 min at 72°C.

After amplification, a total of 20 μ L of each sample (74 total samples) was separated by electrophoresis on a 4% agarose gel (50% agarose and 50% agarose Metaphor, CAMBREX) containing 0.5X TBE buffer (44.5 mM Tris, 44.5 mM boric acid, and 1 mM EDTA). All 72 samples amplified by a single SSR primer were run on the same gel at 60 V for 4 h. A 1-kb ladder (Invitrogen) was used as the weight molecular marker. Gels were stained using 0.5 μ g/mL ethidium bromide, and the image was captured using Molecular Image LOCCUS L-PIX-HE using the Picasa 3 program.

Microsatellite analysis

The allele numbers per locus were determined based on their relative positions in the gel. The presence of a particular allele or peak was given a score of 1, and its absence was given a score of 0. The binary matrix was analyzed using the Popgene 1.32 software (Yeh et al., 1999) and NTSYSpc version 2.1 (Rohlf, 2000). Cluster analysis among the *Saccharum* varieties was based on Jaccard's similarity coefficient using the unweighted pair group method analysis (UPGMA). Genetic variability within each variety of sugarcane was represented by mean H_o . Heterozygous phenotypes for the different alleles were scored within each variety.

RESULTS

Two primers (UGSM148 and UGSM59) were monomorphic for all 74 sugarcane samples analyzed, and the other 15 primers amplified 2 or more products (polymorphic loci). The estimated proportion of polymorphic SSR loci (%P) in the 9 varieties was 88.23%. Seventy alleles, or an average of 4.12 alleles per locus, were detected in the leaves of the 9 varieties of sugarcane plants (Table 3). PCR using primer-specific TA was an efficient strategy because the well-defined extra bands exhibited similar intensities on the gel.

The highest number of alleles (8.0) was detected at the *mSsir56* locus, and the highest gene diversity ($H_o = 1.000$) was detected at the *Smc226CG*, *Smc1237FL*, *Smc1047HA*, *mSsir56*, and *EstB60* loci; the lowest effective number of alleles (1.61) and the lowest gene diversity ($H_o = 0.2838$) were detected at the *Ugsm38*, *Ugsm117*, and *EstB145* loci (Table 3). The mean gene diversity of all SSR loci was 0.7279. The larger effective number of alleles per locus and the higher gene diversity ($H_o > 0.50$) detected in 11 SSR loci (*Segms1069*, *Smc226CG*, *EstB130*, *Smc1237FL*, *Smc165A*, *EstB41*, *Smc1047HA*, *Smc2017FL*, *EstC66*, *mSsir56*, and *EstB60*) suggest that 73.3% of the 15 polymorphic SSR loci are adequate and sufficient to determine differences among the 9 sugarcane varieties.

Table 3. Number of alleles (N_A) and number of effective alleles (N_E) per polymorphic SSR locus and mean observed heterozygosity (H_O) in the 9 sugarcane varieties.

Locus	N	N_A	N_E	H_O
<i>Ugsm38</i>	74	3.0	2.7552	0.2838
<i>Segms240</i>	74	2.0	1.6172	0.4865
<i>Segms1069</i>	74	3.0	2.9104	0.7973
<i>Smc226CG</i>	74	6.0	2.9221	1.0000
<i>EstB130</i>	74	5.0	3.4473	0.9865
<i>Smc1237FL</i>	74	6.0	3.9283	1.0000
<i>Smc165A</i>	74	5.0	2.9883	0.5676
<i>Ugsm117</i>	74	3.0	2.5715	0.2838
<i>EstB41</i>	74	5.0	2.9640	0.6486
<i>EstB145</i>	74	2.0	1.7951	0.2838
<i>Smc1047HA</i>	74	7.0	4.7411	1.0000
<i>Smc2017FL</i>	74	4.0	3.1336	0.8919
<i>EstC66</i>	74	3.0	2.5679	0.6892
<i>mSscir56</i>	74	8.0	3.0212	1.0000
<i>EstB60</i>	74	6.0	2.5904	1.0000
Mean		4.12	2.9302	0.7279

Table 4 shows the proportion of alleles per polymorphic locus and mean H_O in the 9 sugarcane varieties. The number of alleles was highest in the RB72454 sugarcane variety (3.12) and lowest in the RB855453 sugarcane variety (2.29). The RB72454 and RB935744 varieties showed the highest number of polymorphic SSR loci (100%), while the RB835054 variety showed the lowest number of polymorphic SSR loci (75%). The highest H_O value (0.8330) was found in the RB72454 variety, whereas the lowest H_O value (0.5750) was recorded in the SP813250 sugarcane variety. The RB845210 and RB835054 sugarcane varieties also showed lower H_O values (0.6500 and 0.6580, respectively) than the H_O values observed in the RB72454, RB935744, RB867515, RB855453, RB92579, and RB036152 varieties. The SP813250, RB845210, and RB825054 sugarcane varieties were the most genetically uniform varieties.

Table 4. Number of alleles per polymorphic SSR locus (N_A), number of effective alleles (N_E), mean observed homozygosity (Hom_O), mean observed heterozygosity (H_O), and proportion of polymorphic loci (%P) in the plants of the 9 sugarcane varieties.

Variety	N_A	N_E	Hom_O	H_O	(%P)
SP813250	2.823	1.744	0.4250	0.5750	87.50
RB845210	2.823	1.897	0.3500	0.6500	87.50
RB935744	2.94	2.05	0.2580	0.7420	100.00
RB867515	2.47	2.01	0.2080	0.7920	93.75
RB036152	2.35	1.92	0.2670	0.7330	81.25
RB835054	2.59	1.76	0.3420	0.6580	75.00
RB855453	2.29	1.83	0.2670	0.7330	87.50
RB92579	2.59	2.03	0.1920	0.8080	93.75
RB72454	3.12	2.31	0.1670	0.8330	100.00
Total	4.12	2.93	0.2720	0.7280	100.00

An extremely high level of population differentiation (G_{ST}) was detected in samples from all 9 sugarcane varieties ($G_{ST} = 0.7034$), with the G_{ST} for each locus ranging from 0.0578 (*Smc1237FL*) to 1.0000 (*Smc165-1*, *Smc1047HA*, *mSscir56-2*, and *mSscir56-4*). The G_{ST} value indicated that 70.34% of the total variance in allele frequencies at the SSR loci was due to genetic differences among the 9 sugarcane varieties.

The dendrogram obtained from UPGMA cluster analysis of Nei's unbiased genetic distance (Nei, 1973) (Figure 1) revealed the formation of 4 main groups: one group comprising plants from the SP813250 and RB036152 varieties; the second group comprising plants from the RB845210, RB855453, and RB867515 varieties; the third group comprising plants from the RB935744 and RB835054 varieties; and the fourth group comprising the most similar ($I = 0.8631$) RB92579 and RB72454 sugarcane varieties.

Figure 1. Grouping by the unweighted pair group method analysis (UPGMA) based on genetic similarity measures from simple sequence repeat (SSR) loci between the 9 varieties (SP813250, RB036152, RB845210, RB855453, RB867515, RB935744, RB835054, RB92579, and RB72454) of sugarcane.

Groups 2 and 4 had the highest level of genetic similarity compared to plants from the first and third group. Nei's identity values varied between 0.6142 (in the RB92579 and RB935744 sugarcane varieties) and 0.8631 (in the RB92579 and RB72454 sugarcane varieties) (Table 5). The UPGMA dendrogram based on Jaccard's similarity coefficient (Figure 2) represents the genetic variability within each variety of sugarcane. The dendrogram in Figure 2 shows a broad genetic basis (0.45-1.00) for all of the samples analyzed using the 17 SSR loci.

Table 5. Similarity matrix obtained from SSR loci among the 9 varieties (SP813250, RB845210, RB935744, RB867515, RB036152, RB835054, RB855453, RB92579, and RB72454) of sugarcane.

Variety	SP813250	RB845210	RB935744	RB867515	RB036152	RB835054	RB855453	RB92579	RB72454
SP813250	****	0.8069	0.7030	0.7311	0.7717	0.6979	0.7649	0.6869	0.6654
RB845210	0.2146	****	0.7586	0.8160	0.6915	0.6169	0.8288	0.7283	0.7939
RB935744	0.3524	0.2762	****	0.7539	0.7163	0.8049	0.7961	0.6142	0.7330
RB867515	0.3132	0.2034	0.2825	****	0.7213	0.6522	0.8063	0.6703	0.7488
RB036152	0.2592	0.3689	0.3336	0.3268	****	0.6844	0.7182	0.6500	0.7183
RB835054	0.3596	0.4782	0.2171	0.4273	0.3791	****	0.7039	0.6219	0.6797
RB855453	0.2680	0.1878	0.2281	0.2153	0.3310	0.3511	****	0.6638	0.7514
RB92579	0.3756	0.3171	0.4874	0.4000	0.4307	0.4749	0.4098	****	0.8631
RB72454	0.4074	0.2308	0.3106	0.2892	0.3309	0.3861	0.2859	0.1472	****

Figure 2. Grouping by the UPGMA based on genetic similarity measures from SSR loci between the 74 plants of the 9 varieties (SP813250, RB036152, RB845210, RB855453, RB867515, RB935744, RB835054, RB92579, and RB72454) of sugarcane.

DISCUSSION

The adjusted TA for the primers SMC1237FL, SMC1047HA, SMC2017FL, SMC16SA, UGSM59, UGSM38, SEGMS240, SEGMS1069, ESTB130, ESTB41, ESTB145, ESTB60, ESTC66, and mSSCIR56 (Table 2) showed well-defined amplified DNA segments; the faint bands were removed from the gel, and only the segments with similar intensity were counted to avoid overestimating the number of alleles. The number of alleles detected in our study ranged from 2 to 8 per locus, which was similar to what has been reported for other RB sugarcane varieties (Silva et al., 2012) using different SSR primers. The mean number of alleles estimated in our study (4.12 alleles/locus) was also approximately consistent with the mean number of alleles reported in other RB sugarcane varieties (3.2 alleles/locus) (Duarte-Filho et al., 2010), indicating the great potential and high reproducibility of these microsatellite loci to investigate genetic divergence in the sugarcane germplasm. Using a higher number of SSR primers, other breeding programs have reported a correspondingly higher number of alleles at SSR loci (Singh et al., 2008, 2011; Oliveira et al., 2009; Liu et al., 2011).

The genetic diversity within sugarcane varieties estimated from the mean H_o has not been analyzed in other studies with different sugarcane varieties. The analysis of SSR loci in sugarcane has principally been scored based on the genetic distances, where the estimation of the effective number of alleles and the H_o were not calculated (Singh et al., 2008, 2011; Oliveira et al., 2009; Duarte-Filho et al., 2010; Liu et al., 2011; Silva et al., 2012). In our study, the highest heterozygosity detected at SSR loci, e.g., in the RB72454 variety, is potentially meaningful because high heterozygosity would indicate that the plant population likely has a substantial amount of adaptive genetic variation to escape the effects of a control agent that limits the development and maintenance of the plants (Allendorf and Luikart, 2007), in contrast to plant populations showing a lower level of heterozygosity. RB72454 ($H_o = 0.8330$) and RB867515 ($H_o = 0.7920$) are the 2 varieties widely cultivated in Brazil. It is possible that the high rate of polymorphism that is found in these varieties is the factor that favours the establishment of crops, making them less susceptible to problems such as disease and climate variability; therefore, the wide adaptability of these varieties justifies their use in various regions of the country. The RB72454 variety has also been widely used as a parent of other varieties (Table 1) to obtain sugarcane cultivars that develop in the different types of soil and climate that are present in Brazil.

In our study, the RB835054 variety showed the lowest number of polymorphic loci (75%), and the SP813250 genotype had the lowest mean H_o value (0.5700) and the lowest number of alleles ($N_A = 1.74$). Low heterozygosity or high genetic uniformity is considered an important characteristic by industrial sectors when it is associated with morphological and agronomic traits of interest. The genetic uniformity should ensure repeatability in extraction processes, which is an economically important factor for the industrial sector in processing the by-products of sugarcane.

The genetic diversity that was estimated from the dissimilarity matrix among the 9 sugarcane varieties was lower than the genetic diversity that was detected in 30 accessions of other sugarcane varieties using 18 primers. While the genetic similarity among the 9 sugarcane varieties ranged from 0.6242 (RB92579 and RB935744) to 0.8631 (RB92579 and RB72457), the genetic similarity that was estimated in 30 accessions of other RB varieties ranged from 0.56 (RB928054 and RB813904) to 0.95 (RB93509 and RB931011) (Duarte-Filho et al., 2010). The Nei identity values obtained for the 9 sugarcane varieties showed a small genetic basis, which was in contrast to the larger genetic basis reported for other sugarcane accessions. However, a broad genetic basis (0.45 to 1.00) was observed when individual plants of the 9 varieties were compared (Figure 2). A comparison of individual plants showed the genetic variability within each variety of sugarcane. A similarity of 100% for the 17 SSR loci (indicating a clone) was detected only in the SP813250, RB935744, and RB835054 varieties. High and low levels of genetic diversity within other SP and RB varieties have also been reported using random amplification of polymorphic DNA (Silva et al., 2008).

The G_{ST} among sugarcane varieties has not been described in other studies. In this study, the high differentiation among the 9 varieties ($G_{ST} = 0.7034$) suggests the formation of divergent groups. As one of Nei's (1973) genetic diversity statistics, G_{ST} is defined as the proportion of genetic diversity that resides among populations. It is equivalent to Wright's (Wright, 1951) F_{ST} when there are only 2 alleles at a locus, and, in the case of multiple alleles, G_{ST} is equivalent to the weighted average of F_{ST} values for all alleles (Nei, 1973). According to Nei (1973), G_{ST} values higher than 0.25 indicate a very high interpopulational divergence level. Understanding the genetic relationship between individual plants from different groups

may help breeders to select parents when crossing chosen base populations for selection and to maximize heterosis. For example, the RB92579 and RB935744 varieties can be considered as the most genetically divergent varieties for the 17 SSR loci analyzed in our study. The highest G_{ST} was evident at the *Smc16SA*, *Smc1047HA*, and *mSscir56* loci ($G_{ST} = 1.0000$); therefore, the SMC16SA, SMC1047HA, and MSSCIR56 primers are the most adequate primers to characterize the different sugarcane varieties based on the resulting allele frequencies.

Differential levels of genetic variation at SSR loci indicate that particular loci have been the target of artificial selection. Although SSR loci are usually considered just as evolutionarily neutral as DNA markers (Schlötterer and Wiehe, 1999; Schlötterer, 2000), SSR loci of expressed sequence tag-derived SSR markers) have been developed and used for genetic analysis and exploration of the genetic resources of sugarcane (Oliveira et al., 2009). High (*EstB60* and *EstB130* loci), intermediate (*EstB41*), and low (*EstB145* locus) values of genetic diversity (H_o) were detected in the 9 sugarcane varieties analyzed in our study (Table 2). Thus, heterozygous phenotypes at the *EstB60* and *EstB130* loci, as well as at the *Smc226CG*, *Smc1237FL*, *Smc1047HA*, *Smc2017FL*, and *mSsir56* loci ($H_o \geq 0.89$) and favorable alleles at the *Smc16SA*, *Smc1047HA*, and *mSscir56* loci ($G_{ST} = 1.00$), may be targets of artificial selection during the formation of populations, indicating agronomic characteristics that are favorable for sugarcane. The functional significance of a substantial number of SSR loci has been proven by rigorous experiments examining various biological phenomena (Li et al., 2002). Other studies (Subirana and Messeguer, 2008) have indicated that microsatellites with different repeated motifs may be structurally related and involved in the determination of chromosome structure.

Despite the known functional significance of the apparent strong selection detected in some SSR loci, the analysis of genetic structure of the 9 sugarcane varieties using SSR markers was especially important to identify SSR loci with high levels of heterozygosity (*EstB60*, *EstB130*, *Smc226CG*, *Smc1237FL*, *Smc1047HA*, *Smc2017FL*, and *mSsir56*) and varieties showing high heterozygosity (RB72454, RB92579, RB867515, RB935744, RB855453, RB036152), which can then be used as parents to generate or produce new cultivars.

The plants of the RB72454 and RB92579 sugarcane varieties may also be crossed with varieties that present important agronomic characteristics to broaden the genetic basis in sugarcane. Our study with a small number of varieties contradicts previous indications that the germplasm of sugarcane has a narrow genetic base. A broad genetic basis was observed when 8-10 individual plants of each variety were analyzed. The described primers may be used to check the development of each variety, which should be used for the future generation of new hybrid cultivars. The monomorphic primers UGSM148 and UGSM59 are important markers for monitoring the genetic stability of the *Ugsm148* and *Ugsm59* loci. The UGSM148 and UGSM59 primers may be used as molecular markers to compare the genotype stability in the experimental phase of the breeding program while the genotype is evaluated under various environmental conditions. The monomorphic primers may also be used to evaluate the genetic stability of sugarcane during cycles of vegetative multiplication, i.e., propagation via rhizomes.

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