Isozyme diversity in RB (Republic of Brazil) sugarcane (*Saccharum spp*) varieties

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ABSTRACT. Isozyme electrophoresis was used as a method in the identification of biochemical markers for genotype discrimination and for genetic diversity determination in five varieties of sugarcane, *Saccharum* spp. (Poaceae) cultivated in the northern Paraná, Southern Brazil. The five RB (Republic of Brazil) varieties represent improved genetic material, useful for sugar and alcohol production. SOD isozyme pattern can be used as biochemical markers to discriminate the sugarcane RB (72454, 835089, 845257, 855156 and 855536) varieties. The proportion of polymorphic loci was 25% for the RB835089, RB855156, RB855536, and RB72454 varieties and 18.8% for the RB845257 variety. Genetic diversity showed high *I* values; however, the levels of intervariety genetic divergence demonstrated by the *F*-statistic values indicated a deficit of heterozygotes for the *cPer-2* locus ($F_{(IS)}$ = 0.743). The standardized variance in allele frequency among the five RB varieties was $F_{(ST)}$ = 0.434 for the *cPer-2* locus, and the value calculated for the mean standardized variance in allele frequency among the five RB varieties for the 4 loci was $F_{(ST)}$ = 0.182. Thus, the directional selection process for the development of the sugarcane RB varieties seems to have played a major role in determining selection of homozygous plants.

Key words: genetic diversity, isozymes, sugarcane.

RESUMO. Diversidade isozímica em variedades RB (República do Brasil) de cana-de-açúcar (*Saccharum spp*). A eletroforese de isoenzimas foi usada como um método para encontrar marcadores bioquímicos para a identificação de genótipos e para determinar a diversidade genética em cinco variedades de cana-de-açúcar, *Saccharum* spp. (Poaceae) cultivadas na região norte do estado do Paraná, sul do Brasil. As cinco variedades RB (República do Brasil) representam um material genético melhorado, usado para a produção de açúcar e de álcool. O padrão de isoenzimas SOD pode ser usado como um marcador bioquímico para discriminar as variedades RB (72454, 835089, 845257, 855156 e 855536) de cana-de-açúcar. A proporção de *loci* polimórficos foi de 25% para as variedades RB835089, RB855156, RB855536 e RB72454, e de 18.8% para a variedade RB845257. A diversidade genética mostrou valores altos para *I*, entretanto, os níveis de divergência intervariedades demonstrados pelos valores de *F*-estatístico indicaram um *deficit* de heterozigotos para o *locus cPer-2* ($F_{(IS)}$ = 0.743). A variância na freqüência dos alelos entre as cinco variedades RB para o locus *cPer-2* foi $F_{(ST)}$ = 0.434, e o valor calculado para a variância média na freqüência dos alelos para os 4 *loci* entre as cinco variedades RB foi $F_{(ST)}$ = 0.182. Assim, o processo de seleção direcional usado para o desenvolvimento das variedades de cana-de-açúcar RB parece exercer um papel significativo, determinando a seleção de plantas homozigotas.

Palavras-chave: isoenzimas, cana-de-açúcar, diversidade genética, melhoramento genético.

Introduction

Sugarcane, *Saccharum* spp, is a potentially efficient crop in terms of sugar and alcohol production and has been widely cultivated throughout Brazil (Matsuoka *et al.*, 1998). The development of new sugarcane varieties from controlled crosses has been extended and accelerated during recent decades. Therefore, the vast majority of currently available commercial sugarcane varieties have been originated this way.

Controlled crosses between parental varieties (hybridization) can increase the fitness or the adaptive evolution in a number of ways (Arnold, 1997), resulting in new varieties that, because of current preference, are presumably superior to the

ones that are being replaced. The RB (Republic of Brazil) sugarcane varieties represent improved genetic material and are the ones most extensively cultivated in the Southwestern and South regions of Brazil (Matsuoka, 1999).

Botanical and agronomic characteristics have been used for the characterization of different RB sugarcane varieties. Shape, color and dimensions of the thatch, yolk and leaves, leaf scarred, period of maturation (early, mid or end of the milling season), cane production, sucrose content, non-flowering, adaptation to soils, and resistance to the main diseases (leaf scald, mosaic, red stripe, rust, and smut) are the main botanical and agronomic characteristics used by breeders to discriminate the sugarcane RB varieties (Matsuoka *et al.*, 1998). However, the use of morphological and agronomic markers to the selection of crosses between parental RB varieties has, sometimes, conflicted with the breeders' expectation.

Although few investigations have used enzyme electrophoresis for sugarcane genetic studies (Thom and Maretzki, 1970; Nagai *et al.*, 1991; Roughan *et al.*, 1997; Almeida and Crocomo, 1994; D'Hont *et al.*, 1995; Oropeza and Garcia, 1997; Baret *et al.*, 1999; Shoda *et al.*, 1999), starch gel electrophoresis for isozyme analysis is of significant value as a method for determining genetic diversity in plants, because selective neutrality at isozyme loci can be assumed in many cases (Schaal and Olsen, 2000) and, therefore, the isozymes provide a measure of relatedness among the varieties that is not likely to have been directly altered by experimental selection processes. A high level of genetic identity is expected to the RB sugarcane varieties. Thus, the preliminary aim of the current study was the application of the isozyme electrophoresis methodology for identification of specific biochemical markers to discriminate the RB varieties of sugarcane, and to determine the genetic diversity of five sugarcane RB varieties, which have been cultivated for sugar and alcohol production.

Material and methods

The *Saccharum* spp. RB varieties consist of plants traditionally cultivated in the North region of the Paraná State, in southern Brazil. Controlled crosses were performed at the Experimental Station of Serra do Ouro, in the State of Alagoas, northwestern of Brazil. The varieties have been maintained by vegetative propagation at the Ivaí Valley Sugarcane Producers Agricultural Cooperative (Cooperval) to be used for sugar and alcohol production. The varieties evaluated were submitted to electrophoresis for analysis of the superoxide dismutase (SOD; EC 1.15.1.1), malate dehydrogenase (MDH; EC 1.1.1.37), esterase (EST; EC 3.1.1._), and peroxidase (PER; EC 1.11.1.7) isozymes.

There were used samples of young leaves from 10-20 plants from each RB (835089, 845257, 855156, 855536, and 72454) variety of sugarcane. The young leaves (10-20 cm in length) were individually ground to a fine powder in liquid nitrogen and added 80-100 μL of 1.0 *M* phosphate buffer, pH 7.0, containing 5% PVP-40, 0.01 *M* dithiothreitol, 10 m*M* sodium metabisulfite, 50 m*M* ascorbic acid, 1.0 m*M* EDTA, and 0.5% β-mercaptoethanol. After homogenization, the samples were transferred to 1.5 mL microcentrifuge tubes and centrifuged at 14,000 rpm for 30 minutes at 4 °C in a Beckman GS-15R centrifuge.

The supernatants were absorbed with Whatman n°. 3 paper strips (5 x 6 mm), which were inserted vertically into a 14% maize starch gel (penetrose-30) prepared by the procedure originally described by Resende *et al.* (2000) for esterase and peroxidase isozyme analysis.

Malate dehydrogenase and superoxide dismutase isozymes were analyzed using starch gels prepared in 0.05 *M* histidine, 0.0014 *M* EDTA, pH 7.0 adjusted with 1.0 *M* Tris, and the electrode buffer consisted of 0.125 *M* Tris, pH 7.0, adjusted with 1.0 *M* citric acid. Electrophoresis was carried out at 4°C for approximately 15-16 hours, at 2.5 V/cm of gel. For analysis of mitochondrial MDH, the mitochondria were isolated according to the protocol described by Day *et al.* (1985).

The esterase isozymes were visualized by procedures originally described by Hopkinson *et al.* (1973) and Coates *et al.* (1975) and adapted by Resende *et al*. (2000)

MDH isozymes were stained with the reaction mixture reported by Machado *et al.* (1993), and SOD isozymes were visualized as achromatic regions after 60 min. on the same gel used to develop the MDH isozymes, or when the gels were incubated with only 15 ml 0.1 *M* Tris/HCl buffer, pH 8.6, 0.5 ml MTT, 0.5 ml PMS, and 15 ml 2% agar.

The genetic variability and genetic structure of the RB sugarcane varieties under study was analyzed using the BIOSYS-1/A Computer Program for the Analysis of Allelic Variation in Genetics (Swofford and Selander, 1981). Allele frequencies, mean heterozygosity and mean number of alleles per locus, percentage of polymorphic loci, and genetic identity were calculated for the five RB varieties of *Saccharum* spp. The genetic structure of populations was analyzed using *F*-statistics (Wright, 1965), i.e.,

the level of interpopulational genetic divergence (F_{ST}), level of inbreeding (F_{IT}), and deficit of heterozygous device to nonrandom mating (F_{IS}).

Results

The isozyme patterns of the RB varieties of sugarcane showed a differential SOD isozyme pattern for each variety, but there were no distinct SOD isozyme phenotype variants in plants within each variety (Figure 1). Anodal (aSOD) and cathodal SOD (cSOD) isozymes were numbered in increasing order from the gel origin. The cSOD-1 and aSOD-2 isozyme were observed in all plants analyzed; aSOD-1, and aSOD-3 were detected in plants of the RB72454, RB855536, and RB845257 varieties; aSOD-4 isozyme was detected in plants of the RB855536 and RB72454 varieties, and aSOD-5 isozyme was observed in plants of the RB72454, RB855536, and RB845257 varieties.

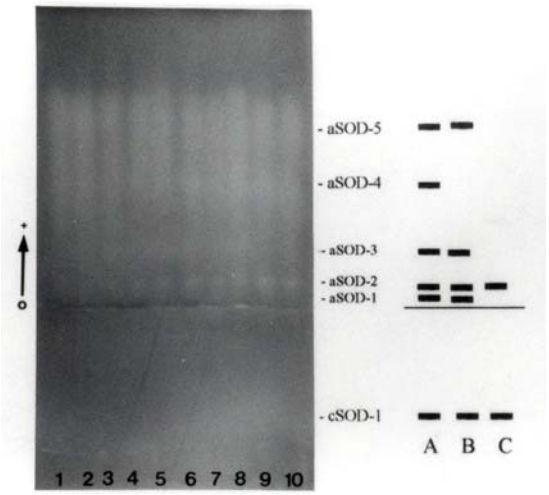

Figure 1. Cathodal (c) and anodal (a) superoxide dismutase (SOD) isozymes obtained in Tris/histidine/EDTA, pH 7.0, for young leaves of the RB72454 variety (left) of *Saccharum* spp. (samples 1-10), and electrophoretic phenotypes (right) shown by the RB855536 (A), RB845257 (B), and RB835089 (C) varieties of sugarcane

Malate dehydrogenase isozyme analysis revealed a similar pattern in all plants of the RB varieties. The faster anodal region was considered to be the cytoplasmic MDH isozyme (sMDH) while mitochondrial analysis showed that the mtMDH-1, mtMDH-2 and mtMDH-3 isozymes were of mitochondrial origin (Figure 2). Cytoplasmic MDH isozymes showed that the sMDH-1/sMDH-2/sMDH-3 electrophoretic phenotype indicates the presence of two *sMdh* loci and a dimeric structure for the MDH isozymes. The slower region of the MDH isozyme phenotype (Figure 2) was considered to be the microbody isozyme (mbMDH), in agreement with data reported for most plants (Newton, 1983; Machado *et al.*, 1993; Jorge *et al.*, 1997; Resende *et al.*, 2000). Different mbMDH electrophoretic phenotypes were observed in plants of the RB varieties, but the pattern observed indicated no allelic variation. The different mbMDH isozymes seem to be the consequence of a differential metabolic activity status of the plants and should reflect the different expression of genetic information and regulation of different gene activities, which need further investigation.

Allelic variations were detected at three of the five PER isozyme loci and at one of the EST isozyme loci. Two alleles were identified for each cathodal *cPer-1* (*cPer-1*^{1} and *cPer-1*^{2}) and *cPer-2* (*cPer-2*^{1} and *cPer-2*^{2}) locus, and three alleles were detected for the *aPer-1* (*aPer-1*^{1}, *aPer-1*^{2} and *aPer-1*^{3}) locus in the RB835089 and RB855636 varieties (Table 1, Figure 3).

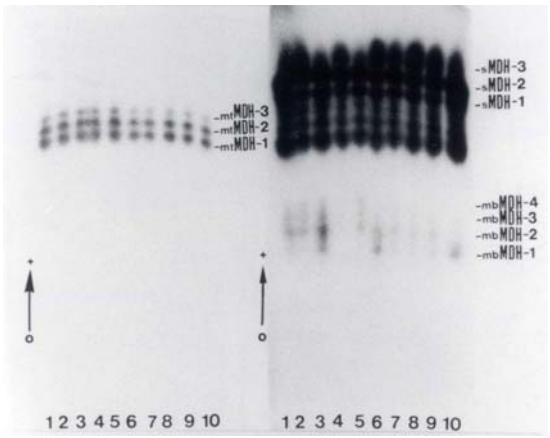

Figure 2. Malate dehydrogenase isozyme patterns obtained in Tris/histidine/EDTA, pH 7.0, for young leaves of the RB72454 (samples 1-5) and RB855156 (samples 6-10) varieties of *Saccharum* spp. (Left) Mitochondrial MDH isozymes (mtMDH-1, mtMDH-2, and mtMDH-3) obtained by mitochondrial isolation procedures (Day *et al.*, 1985). (Right) Cytosol MDH isozymes (sMDH-1, sMDH-2, and sMDH-3) and microbody MDH isozymes (mbMDH-1, mbMDH-2, mbMDH-3, and mbMDH-4)

The esterase isozyme patterns, all showing anodal migration (Figure 4) indicated three loci (*Est-1*, *Est-2* and *Est-6*) when 4-methylumbelliferyl acetate was used as substrate, two loci (*Est-3* and *Est-4*) when 4-methylumbelliferyl propionate was used, and four loci (*Est-1*, *Est-5*, *Est-6*, *Est-7*) when α-naphthyl acetate and α-naphthyl propionate were used. Substrate preference of esterase isozyme was observed since the EST-3 and EST-4 isozyme were

observed only with 4-methylumbelliferyl propionate, and the products of *Est-2* locus, i.e., the product of three alleles *Est-2*^{1}, *Est-2*^{2} and *Est-2*^{3} were observed only with 4-methylumbelliferyl acetate. The three *Est-2* alleles were detected in all five RB varieties (Table 1).

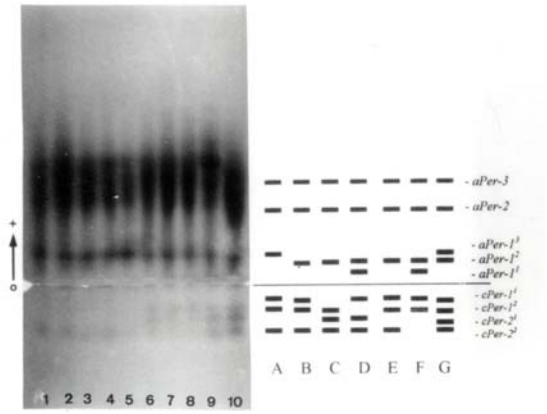

Figure 3. Cathodal (c) and anodal aisoperoxidases (PER) obtained in Tris/citrate, pH 7.5, for young leaves of sugarcane (*Saccharum* spp.). (Left) samples 1, 4, and 5 correspond to the cPER-1$^{1/2}$/cPER-2$^{2/2}$/aPER-1$^{3/3}$ phenotype and samples 2 and 3 correspond to the cPER-1$^{1/2}$/cPER-2$^{2/2}$/aPER-1$^{2/2}$ phenotype detected in the RB855156 variety; and samples 6-10 correspond to the cPER-1$^{2/2}$/cPER-2$^{1/2}$/aPER-1$^{2/2}$ phenotype detected in the RB855536 variety. (Right) Different PER isozyme phenotypes: cPER-1$^{1/2}$/cPER-2$^{2/2}$/aPER-1$^{3/3}$ (A), cPER-1$^{1/2}$/cPER-2$^{2/2}$/aPER-1$^{2/2}$ (B), cPER-1$^{2/2}$/cPER-2$^{1/2}$/aPER-1$^{2/2}$ (C), cPER-1$^{1/1}$/cPER-2$^{1/2}$/aPER-1$^{1/2}$ (D), cPER-1$^{1/2}$/cPER-2$^{2/2}$/aPER-1$^{2/2}$ (E), cPER-1$^{1/1}$/cPER-2$^{1/1}$/aPER-1$^{1/2}$ (F), cPER-1$^{1/2}$/cPER-2$^{1/2}$/aPER-1$^{2/3}$ (G) shown by the RB varieties

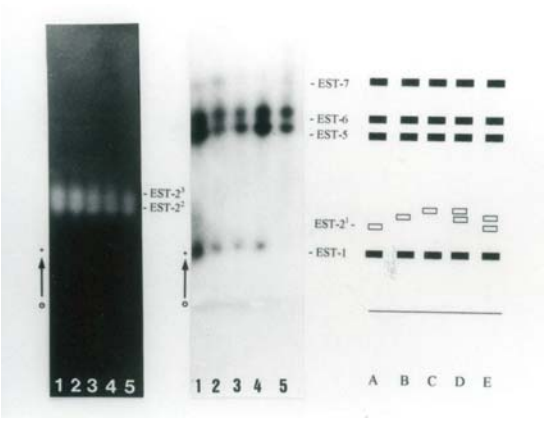

Figure 4. Esterase isozymes obtained in Tris/citrate, pH 7.5, for young leaves of *Saccharum* spp. Samples 1-5 correspond to plants of the RB72454 sugarcane variety showing the *Est-2*$^{2/3}$ genotype observed using 4-methylumbelliferyl acetate (left), and the EST-1, EST-5, EST-6, and EST-7 isozymes observed using α-naphthyl acetate (center). (Right) Isoesterase phenotypes EST-1/EST-2^1/EST-5/EST-6/EST-7 (A), EST-1/EST-2^2/EST-5/EST-6/EST-7 (B), EST-1/EST-2^3/EST-5/EST-6/EST-7 (C), EST-1/EST-2$^{2/3}$/EST-5/EST-6/EST-7 (D), and EST-1/EST-2$^{1/2}$/EST-5/EST-6/EST-7 (E) detected in the five RB varieties using the two substrates

Thus, the isozyme patterns of the sugarcane RB varieties indicated a total of 4, 5 and 7 loci in the MDH, PER and EST isozymes, respectively. Allele frequencies for each RB variety, mean heterozygosity per locus and effective number of alleles per locus of the five RB varieties are listed in Table 1. The proportion of polymorphic loci was 25% for the RB835089, RB 855156, RB855536, and RB 72454 varieties and 18.8% for the RB845257 variety (Table 1). SOD isozymes were not computed in the analysis of genetic identity, since the numbers of loci and alleles for these isozymes was not determined.

Chi-square analysis showed no differences between observed genotypic frequencies and expected Hardy-Weinberg frequencies for the *cPer-1* locus of the RB 835089, RB 8551556, RB 855536 and RB 72454 varieties, for the *cPer-2* locus of the RB 855536 and RB 72454 varieties, for the *aPer-1* locus of the RB835089, RB 855536, and RB 72454 varieties, and for the *Est-2* locus of the RB 72454 variety (Table 2). Deviations from the expected equilibrium were observed in the RB 835089 variety for the *cPer-2* and *Est-2* loci, in the RB 855156 variety for *cPer-2*, *aPer-1* and *Est-2* loci, in the RB 845257 variety for the *cPer-1*, *aPer-1*, and *Est-2* loci, and in the RB 855536 variety for the *Est-2* locus. Heterozygote deficiency, represented by the fixation index (*F*) given in Table 2, showed higher values of *F* at the *cPer-1* locus of the RB 845257 variety, at the *cPer-2* locus of the RB 835089 and RB 855156 varieties, at the *aPer-1* locus of the RB 855156 and RB 845257 varieties, and at the *Est-2* locus of the RB 835089 and RB 855156 varieties.

Genetic diversity calculated by Nei's (1972) genetic identity (*I*) showed high *I* values for the five sugarcane RB varieties (Table 3).

The levels of intervariety genetic divergence are demonstrated by the $F_{(ST)}$ (Table 4), and the *F*-statistic (Wright, 1965) values calculated for the five sugarcane RB varieties indicated a deficit of heterozygotes for the *cPer-2* locus ($F_{(IS)}$ = 0.743). The standardized variance in allele frequency among the five RB varieties for the *cPer-2* locus was $F_{(ST)}$ = 0.434, and the $F_{(ST)}$ = 0.182 was the value calculated for the mean standardized variance in allele frequency among the five RB varieties for the 4 loci.

Table 1. Allele frequencies and mean heterozygosities for the cPer-1, cPer-2, aPer-1, and Est-2 loci in RB(835089, 855156, 845257, 855536, 72454) varieties of *Saccharum* spp

	RB Varieties															
	835089				855156				845257				855536				72454			
Locus	*cPer-1*	*cPer-2*	*aPer-1*	*Est-2*	*cPer-1*	*cPer-2*	*aPer-1*	*Est-2*	*cPer-1*	*cPer-2*	*aPer-1*	*Est-2*	*cPer-1*	*cPer-2*	*aPer-1*	*Est-2*	*cPer-1*	*cPer-2*	*aPer-1*	*Est-2*
Allele																				
1	0,250	0.800	0.100	0.100	0.846	0.308	0.000	0.192	0.667	0.000	0.000	0.100	0.250	0.625	0.075	0.100	0.625	0.042	0.000	0.125
2	0.750	0.200	0.800	0.700	0.154	0.692	0.692	0.538	0.333	1.000	0.633	0.500	0.333	1.000	0.633	0.500	0.375	0.958	0.417	0.583
3	0.000	0.000	0.100	0.200	0.000	0.000	0.308	0.269	0.000	0.000	0.367	0.400	0.000	0.000	0.250	0.400	0.000	0.000	0.583	0.292
Mean Heterozygosity																				
Ho	0.056				0.029				0.067				0.131				0.089			
He	0.098				0.111				0.096				0.122				0.104			
Ho / Locus	0.014				0.007				0.016				0.033				0.022			
He / Locus	0.025				0.028				0.024				0.031				0.026			
Mean number of alleles/locus = 1.4					1.3				1.3				1.4				1.3			
% Polymorphic loci = 25					25				18.8				25				25			

Ho: observed mean heterozygosity; *He*: expected mean heterozygosity

Table 2. Comparison of genotypic frequencies for the *cPer-1*, *cPer-2*, *aPer-1*, and *Est-2* loci in RB(835089, 855156, 845257, 855536, 72454) varieties of *Saccharum* spp

	RB Varieties																				
	835089					855156				845257				855536				72454			
Genotype	O.N.	E.N.	χ^2	df	*F*	O.N.	E.N.	χ^2	df	*F*	O.N.	E.N.	χ^2	df	*F*	O.N.	E.N.	χ^2	df	*F*	O.N.	E.N.	χ^2	df	*F*
cPer-1$^{1/1}$	0	0.526				9	9.240				10	6.552				0	1.154				6	4.565			
cPer-1$^{1/2}$	5	3.947				4	3.520				0	6.897				10	7.692				3	5.870			
cPer-1$^{2/2}$	5	5.526				0	0.240				5	1.552				10	11.154				3	1.565			
			0.079^{ns}	1				0.000^{ns}	1				12.861*	1				0.834^{ns}	1				1.786^{ns}	1	
					-0.333					-0.182					1.000					0.333					0.467
cPer-2$^{1/1}$	8	6.316				4	1.120									10	7.692				0	0.000			
cPer-2$^{1/2}$	0	3.368				0	5.760									5	9.615				1	1.000			
cPer-2$^{2/2}$	2	0.316				9	6.120									5	2.692				11	11.000			
			7.105*	1				10.786*	1									3.40^{ns}	1				0.000^{ns}	1	
					1.000					1.000					0.467										-0.043
aPer-1$^{1/1}$	0	0.053														0	0.077								
aPer-1$^{1/2}$	2	1.684														3	2.077								
aPer-1$^{1/3}$	0	0.211														0	0.769								
aPer-1$^{2/2}$	6	6.316				9	6.120				9	5.897				10	9.000				2	1.957			
aPer-1$^{2/3}$	2	1.684				0	5.760				1	7.207				4	6.923				6	6.087			
aPer-1$^{3/3}$	0	0.053				4	1.120				5	1.897				3	1.154				4	3.957			
			0.000^{ns}	3				10.786*	3				9.242*	3				2.627^{ns}	3				0.000^{ns}	3	
					-0.176					1.000					0.856					0.265					-0.029
Est-2$^{1/1}$	1	0.053				2	0.400				0	0.103				0	0.154				1	0.130			
Est-2$^{1/2}$	0	1.474				1	2.800				3	1.552				4	2.051				1	1.826			
Est-2$^{1/3}$	0	0.421				0	1.400				0	1.241				0	1.641				0	0.913			
Est-2$^{2/2}$	7	4.789				6	3.640				0	3.621				0	4.872				4	3.957			
Est-2$^{2/3}$	0	2.947				1	3.920				12	6.207				16	8.205				5	4.261			
Est-2$^{3/3}$	2	0.316				3	0.840				0	2.276				0	3.077				1	0.913			
			11.530*	3				9.932*	3				9.612*	3				14.383*	3				1.306^{ns}	3	
					1.000					0.744					-0.724					-0.724					0.106

O.N.: observed number of plant; E.N.: expected number of plants; ns: Not significant ($P > 0.01$); * Significant ($P < 0.01$); *F*: Fixation index for polymorphic loci

Table 3. Genetic identity (Nei, 1972) between the RB (835089, 855156, 845257, 855536, 72454) varieties of *Saccharum* spp

RB Variety	835089	855156	845257	855536	72454
835089	-	0.960	0.942	0.998	0.941
855156	-	-	0.994	0.971	0.991
845257	-	-	-	0.963	1.000
855536	-	-	-	-	0.963
72453	-	-	-	-	-

Table 4. Genetic divergence between the RB (835089, 855156, 845257, 855536, 72454) varieties of *Saccharum* spp

Locus	$F_{(IS)}$	$F_{(IT)}$	$F_{(ST)}$
cPer-1	0.190	0.375	0.228
cPer-2	0.743	0.854	0.434
aPer-1	0.400	0.453	0.089
Est-2	0.045	0.067	0.023
Mean	**0.276**	**0.408**	**0.182**

F: fixation values (Wright, 1965)

Discussion

Although MDH isozyme patterns have been used as markers to identify sugarcane cultivars (Oropeza and Garcia, 1997), in the present study a monomorphic pattern of sMDH and mtMDH isozymes was observed within and between the RB varieties. The differential mbMDH isozyme expression observed in plants of the RB varieties could be the consequence of a differential metabolic status of the plants, since differential activities of MDH isozymes have been reported for different sugarcane varieties in different temperatures (Du *et al.*, 1999). High activity of MDH isozymes and a differential response to the period of exposure to light have also been reported for *Saccharum officinarum* (Grotjohann *et al.*, 2000).

The segregation distortion observed for the *cPer-1* (RB845257), *cPer-2* (RB835089, RB855156), *aPer-1* (RB855156, RB845257), and *Est-2* (RB835089, RB855156, RB845257, and RB855536) loci in the different varieties may be attributed to the method used to establish each of the five RB varieties. The selection of plants with characteristics of agronomic interest and their clonal multiplication are events that can produce nonrandom phenotypic distribution and the absence of Hardy-Weinberg equilibrium. The RB 72454 variety is usually employed as the parental genotype in most of the hybridization processes for obtaining different sugarcane varieties (Matsuoka *et al.*, 1998; Matsuoka, 1999). This variety has been used as a male or female parent for the production of important commercial sugarcane varieties. Distortion in the segregation patterns for some esterase and peroxidase isozymes was also observed in Japanese varieties of sugarcane (Shoda *et al.*, 1999).

The level of genetic variability found in the RB varieties was lower (18% and 25%) than the mean proportion value (38.8%) reported for a group of different plant species (Hamrick, 1989), but it was similar to the values reported for continental populations (Northeastern Brazil) of colonizing plants (Barrett and Shore, 1989). The number of alleles per locus observed was similar (1.3 and 1.4) to the value reported for dicotyledons (1.46) and the observed proportion of heterozygous loci was higher than the values reported for colonizing plants (Barrett and Shore, 1989) only in the RB 855536 and RB 72454 varieties.

The high genetic identity (*I*) of the RB varieties showed that, in terms of electrophoretically detectable genetic variation, the plants of the RB varieties are closely inter-related. Thus, considerable morphological divergence can occur in the five different varieties (Matsuoka *et al.*, 1998), with little or no differentiation in allozymes. RAPD markers for the study of molecular diversity in the *Saccharum* complex (*S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barberi*, *S. sinense*, and *Erianthus* ssp.) have shown that *S. officinarum* have a low level of genetic diversity (Nair *et al.*, 1999).

On the other hand, the values of Wright's $F_{(ST)}$ statistic for the *cPer-1* (0.228) and *cPer-2* (0.434) loci seem to indicate an increase in the RB varieties differentiation. Usually, artificial selection process should increase population differentiation as would genetic drift (Hamrick, 1989). The $F_{(IS)}$ values calculated for the *cPer-2* (0.743) and *aPer-1* (0.40) loci also reflect a deficit of heterozygotes, produced by directional selection to obtain plants with the best phenotypes for the characteristics of agronomic interest. Based on high mean $F_{(IT)}$ value (0.408) and high $F_{(IT)}$ values for the *cPer-1* (0.375), *cPer-2* (0.854), and *aPer-1* (0.453) loci, the selection process also induces subdivision of RB varieties into populations. Thus, the directional selection process for the development of the sugarcane RB varieties seems to have played a major role in determining the genetic structure of these RB variety populations.

Natural selection usually favors highly heterozygous individuals and most cases of selection for viability in plant populations reveal advantages for heterozygotes (Mitton, 1989). Although these observations are not universal, highly heterozygous individuals often have superior growth rates and developmental stability, compared to highly homozygous individuals from the same populations. A relationship between protein heterozygosity and growth has been described in different plant species. Analysis of additional isozyme loci or others molecular markers may be performed to confirm the selection of homozygous plants in RB varieties of sugarcane indicated by the present data. Although isozyme variations may not yet be directly used to predict superior field performance, they reflect the constitutive gene expression, and can provide valuable data as markers for selection of genotypes that are relevant to sugarcane genetic breeding. The present data show that the SOD isozyme pattern can be used as biochemical markers to discriminate the sugarcane RB (72454, 835089, 845257, 855156 and 855536) varieties. In addition, plants showing a distant relationship (lower I values) may be recommended as parental generation for breeding programs implementation.

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