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Genetic variability among sugarcane genotypes based on polymorphisms in sucrose metabolism and drought tolerance genes

Silvana Creste · Klaus A. G. Accoroni · Luciana R. Pinto · Roland Vencovsky · Marcos A. Gimenes · Mauro A. Xavier · Marcos G. A. Landell

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Abstract Target region amplification polymorphism (TRAP) markers were used to estimate the genetic similarity (GS) among 53 sugarcane varieties and five species of the *Saccharum* complex. Seven fixed primers designed from candidate genes involved in sucrose metabolism and three from those involved in drought response metabolism were used in combination with three arbitrary primers. The clustering of the genotypes for sucrose metabolism and drought response were similar, but the GS based on Jaccard's coefficient changed. The GS based on polymorphism in sucrose genes estimated in a set of 46 Brazilian varieties, all of which belong to the three Brazilian breeding programs, ranged from 0.52 to 0.9, and that based on drought data ranged from 0.44 to 0.95. The results suggest that genetic variability in the evaluated genes was lower in the sucrose metabolism genes than in the drought response metabolism ones.

Keywords Functional markers · Genetic diversity · Genetic resources

Abbreviations

Aqua	Aquaporin
Arb	Arbitrary primer
DBF	Dehydration binding factor
DirH	Dirigent protein related to high sugar content
DirL	Dirigent protein related to low sugar content
LEA	Late embryogenesis abundant protein
PIC	Polymorphism information content
SAI	Soluble acid invertase
SPS	Sucrose phosphate synthase
Susy	Sucrose synthase
Sut4 and Sut	Sugar transporters
TRAP	Target region amplified polymorphism

Introduction

Sugarcane is one of the most important crops in Brazil and is the main source of raw material for the production of sugar and bioethanol. As such, it is

S. Creste (✉) · K. A. G. Accoroni · L. R. Pinto · M. A. Xavier · M. G. A. Landell
Centro de Cana, Instituto Agronômico de Campinas, Anel Viário Contorno Sul, Km 321, CP 206, Ribeirão Preto, SP 14001-970, Brazil
e-mail: screste@iac.sp.gov.br

R. Vencovsky
Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Pádua Dias, 11, CP 83, Piracicaba, SP 13400-970, Brazil

M. A. Gimenes
Embrapa Recursos Genéticos e Biotecnologia (CENARGEN), SAIN, PqEB, CP 02372, Brasília CEP 70770-900, DF, Brazil

essential to the economy and well-being of Brazil. At least two *Saccharum* species are involved in its breeding history (Daniels and Roach 1987; Sreenivasan et al. 1987). The progenies obtained were repeatedly backcrossed with *S. officinarum* clones to recover the favorable alleles for sugar content from the recurrent parent; this process is referred to as "nobilization" (Roach 1972).

The very few first hybrids obtained were extensively intercrossed, generating hundreds of sugarcane varieties that in turn determined a very narrow genetic base for the sugarcane crop (Selvi et al. 2006). Despite the breakthrough in sugarcane improvement, an apparent plateau has been reached in terms of sugar concentration, and selection for new higher yielding varieties has proven to be difficult (Ming et al. 2002). One stark consequence of this plateau is that the increment achieved to date will not be adequate to meet the Brazilian sugar and ethanol demand that has been projected for the next decade. For this reason, it is extremely important to quantify the amount of genetic variation that is presents in the germplasm that has been exploited as the parents in sugarcane breeding programs in Brazil.

Molecular markers are important tools in breeding programs since they enable the genetic distance (GD) between genotypes to be estimated. In terms of sugarcane germplasm, the genetic variability has been estimated using different molecular markers, such as random fragment length polymorphism (RFLP; D'Hont et al. 1994; Janoo et al. 1999; Coto et al. 2002; Schenck et al. 2004), ribosomal DNA (Glaszmann et al. 1990), microsatellites (Piperidis et al. 2000; Pan et al. 2003; Cordeiro et al. 2003; Pinto et al. 2006;), amplified fragment length polymorphism (AFLP; Hoarau et al. 2002; Lima et al. 2002; Aitken et al. 2006; Selvi et al. 2006). Most of these markers reflect the genetic variability in non-coding or repetitive DNA regions of the genome.

Information on genetic variability may contribute to germplasm conservation and use, such as by identifying duplicates in databanks, in monitoring the genetic integrity of accessions, and by providing extra information that may help in the establishment of relationships between genotypes. Traditionally, the choice of parents in sugarcane breeding programs has been defined on the basis of agronomic characters and pedigree records, using bi-parental crosses or polycrosses between elite genotypes. However, the lack of genealogy data as well as the inadequate identification of some genotypes have not enabled an accurate estimation of the GD among sugarcane genotypes based on pedigree data. In addition, the continuous selection for the same traits in breeding programs, such as sucrose content, has caused a reduction in genetic diversity, limiting further advances in sugarcane breeding.

It has been suggested that the measure of genetic diversity by molecular markers for breeding purposes should be based on functionally characterized genes, or targeted genes, as these may reflect functional polymorphisms (Andersen and Lübberstedt 2003; Ramalingam et al. 2003). The variation in expressed or regulatory sequences may reflect the past influences of selections, which could be different for each gene. For example, the characteristics that enable a sugarcane variety to adapt to a specific environment may depend on a limited set of genes, and the variation in such genes will probably not be the same as that in a group of genes involved in the expression of an independent characteristic. Therefore, the variation in those genes most probably will not be detected using random markers (Tienderen et al. 2002). For this reason, it has been suggested that genetic diversity estimation for planning crossing purposes should be done based on candidate genes for specific traits (Tienderen et al. 2002; Andersen and Lübberstedt 2003; Liu et al. 2004; Alwala et al. 2006).

Many techniques are currently available for assaying genetic variation in genes. The target region amplification polymorphism (TRAP) technique allows the identification of polymorphisms in coding regions using one fixed primer designed from a target expressed sequence tag (EST) sequence and a second random primer of arbitrary sequence except for AT- or GC-rich cores that anneal with introns and exons, respectively (Hu and Vick 2003).

We have exploited the potential of the TRAP technique to estimate the genetic similarity (GS) among a set of sugarcane varieties used as parents in Brazilian sugarcane breeding programs. The polymorphism in sucrose metabolism and drought tolerance genes was evaluated in a set of seven and three candidate genes, respectively. The results of this study are reported here.

Materials and methods

Plant material

A total of 60 sugarcane genotypes comprising 53 varieties and five species of the *Saccharum* complex (three accessions of *S. officinarum*, one *S. spontaneum*, one *S. barberi*, one *S. robustum*, and one *Erianthus*), maintained at the collection of "Centro de Cana, Instituto Agronômico de Campinas—(IAC)", SP, Brazil were evaluated. The varieties were chosen based on their economic importance in Brazilian sugarcane production areas or on their importance as parents for crosses. The species were chosen so as to be able to check the potential of TRAP markers to establish genetic relationships: two species (*S. oficinarum* and *S. spontaneum*) are closely related to cultivated sugarcane and *Erianthus* sp, is a genus related to *Saccharum*. The genotypes with their respective parents, the Pol value (Pol), and drought response (level of drought tolerance), where known, are presented in Table 1.

PCR primers design

Two groups of fixed primers were used in this study (Table 2). The first group comprised seven primers designed based on sucrose metabolism sequence genes, mainly from principal metabolism (sucrose synthase, Susy; sucrose phosphate synthase, SPS; soluble acid invertase, SAI), candidate genes encoding for sugar transporters (Sut4 and Sut), and dirigent proteins previously identified in differential expression analysis experiments in plants with high (DirH) and low (DirL) sucrose content. The second group comprised three fixed primers designed from candidate genes involved in the drought tolerance response metabolism (Aquaporin, Aqua; late embryogenesis abundant protein, LEA; dehydration binding factor, DBF). The LEA and Aqua candidate genes belong to gene families, respectively, and the primers were designed based on conserved regions. The Clustal X ver.1.81 software program (Thompson et al. 1997) was used to align the sequences. For both groups, primers were designed on the basis of the web-based PCR primer design software Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) using the following parameters: a primer optimal T_m, maximum T_m, and minimum T_m of 53, 55 and 50°C, respectively, and a GC content between 40 and 60%. The sequence information of three arbitrary primers used in this study was provided by Li and Quiros (2001). The primer details are described in Table 2.

DNA extraction and PCR cycling conditions

Total DNA was extracted from a fresh meristem cylinder (Al-Janabi et al. 1999). All PCR reactions were performed in a Bio-Rad Myclycler Termalcycler (Hercules, CA) in 13-µl reactions containing 60 ng DNA sample, 1.3 µl 10× reaction buffer (Biotools B&M Labs, Spain), 0.65 µl 50 mM $MgCl_2$, 0.26 µl 10 mM dNTPs, 0.2 µM of each primer, and 0.5 Us of *Taq* DNA polymerase (Biotools B&M Labs). Amplifications were performed by initially denaturing the template DNA at 94°C for 2 min, followed initially by five cycles at 94°C for 45 s, 35°C for 45 s, and 72°C for 1 min and then by 35 cycles at 94°C for 45 s, 50°C for 45 s, and 72°C for 1 min, and terminated with a final extension step at 72°C for 7 min.

Electrophoresis and polymorphism detection

Sequencing gels (6% polyacrylamide, 8 M urea) were run under standard conditions, and the PCR products were visualized by silver staining (Creste et al. 2001).

Data analysis

Amplified fragments were scored for presence or absence in all 60 genotypes. The GS among all genotypes were calculated using Jaccard's similarity coefficient: $GS_{ij} = a/(a + b + c)$, where GS_{ij} is the measurement of the GS between individuals i and j, a is the number of polymorphic bands present in both individuals, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. Relationships among genotypes were evaluated with phenetic cluster analysis using the unweighted pair-grouping with arithmetic average (UPGMA) clustering and then plotted in a phenogram using NTSYS-PC ver. 2.0j (Exeter Software, Setauket, NY). The extent to which the dendrogram represented the original distance matrices was assessed by first calculating a matrix of cophenetic

Table 1 Sugarcane genotypes evaluated by TRAP markers derived from sucrose and drought response metabolism genes

Genotype	Female parent	Male parent[a]	Pol[b]	Drought response[c]
(1) IAC86-3154	CP5248	CO798	12.05	Sensitive
(2) IAC91-4168	SP813137	?	12.62	Sensitive
(3) IAC91-2205	RB855035	?	15.93	Sensitive
(4) IAC48-65	SP813137	?	15.00	Tolerant
(5) IAC91-3093	SP801520	?	14.76	Sensitive
(6) IAC87-3396	CO740	SP701143	15.86	Tolerant
(7) IAC91-2195	RB785148	?	16.34	Sensitive
(8) IAC95-5011	SP81520	SP80-1842	12.05	Sensitive
(9) IAC91-5155	SP803212	?	16.04	Sensitive
(10) IAC91-2218	RB855035	?	16.15	Sensitive
(11) IAC86-2480	US71399	?	16.29	Tolerant
(12) IAC86-2210	CP5248	Co798	16.38	Tolerant
(13) IACSP95-2078	SP80185	?	16.97	Sensitive
(14) IACSP95-5000	SP80-2066	SP80-185	16.09	Tolerant
(15) IACSP95-2288	SP803280	RB835486	16.47	Sensitive
(16) IACSP95-5037	SP842189	SP801842	14.11	Sensitive
(17) IACSP95-6114	IAC873187	CTC9019	15.75	Sensitive
(18) IACSP95-3018	SP842189	SP801842	16.22	Sensitive
(19) IACSP93-3046	SP791011	?	16.40	Tolerant
(20) IACSP96-2022	SP84-5019	IAC82-3092	17.19	Sensitive
(21) IACSP93-6006	SP791011	?	15.90	Sensitive
(22) IACSP96-2019	SP81-3251	SP84-1182	14.21	Sensitive
(23) IACSP94-4004	SP82-6108	SP775181	15.35	Tolerant
(24) IACSP94-2101	SP775181	RB785148	16.17	Tolerant
(25) IACSP95-2048	SP82-6108	SP80-3280	16.03	Sensitive
(26) IACSP94-2094	SP84-7017	?	16.25	Tolerant
(27) SP80-4966	SP71-1406	?	16.60	Sensitive
(28) SP80-180	B3337	?	14.09	Sensitive
(29) SP84-7017	CP57542	?	15.30	Sensitive
(30) SP79-1011	NA5679	CO775	16.23	Tolerant
(31) SP70-1143	IAC48-65	?	15.05	Tolerant
(32) SP80-3280	SP711088	H575028	15.92	Sensitive
(33) SP84-1201	CO62175	?	15.05	Sensitive
(34) SP91-1049	SP803328	SP813250	17.78	Sensitive
(35) SP90-3414	SP801079	SP823544	15.12	Sensitive
(36) SP83-2847	HJ5741	SP701143	14.01	Sensitive
(37) SP89-1115	CP731577	?	15.89	Sensitive
(38) SP91-3011	RB84257	?	16.25	Sensitive
(39) SP86-42	SP70-1143	?	16.03	Tolerant
(40) RB72-454	CP5376	?	15.67	Sensitive
(41) RB85-5156	RB72454	TUC717	14.77	Sensitive
(42) RB83-5486	L6014	?	16.46	Tolerant
(43) RB85-5595	SP70-1143	TUC717	16.40	Sensitive
(44) RB92-8064	SP701143	?	15.28	Sensitive

Table 1 continued

Genotype	Female parent	Male parent[a]	Pol[b]	Drought response[c]
(45) RB86-7515	RB72454	?	15.44	Tolerant
(46) RB85-5453	TUC717	?	16.78	Sensitive
(47) CO 419	POJ2878	CO290	–	–
(48) CO 997	CO683	P16332	–	–
(49) CO 740	P3247	P4775	–	–
(50) NA56-79	CO419 (self)		–	–
(51) POJ 2878	POJ2364	EK28	–	–
(52) HJ 5741	H401184	?	–	–
(53) R 570	H393633	M20246	–	–
(54) *Saccharum officinarum* 82-72	–	–	–	–
(55) *S. officinarum* 82-80	–	–	–	–
(56) White transparent (*S. officinarum*)	–	–	–	–
(57) SES 205 A (*S. spontaneum*)	–	–	–	–
(58) US 5714 105 (*S.robustum*)	–	–	–	–
(59) Chunnee (*S. barberi*)	–	–	–	–
(60) 75 II 09 *Erianthus*	–	–	–	–

[a] ?, Unknown parental derived from polycrosses

[b] Average Pol values were obtained from IAC ("Centro de Cana, Instituto Agronômico de Campinas") experimental network assays, taking as reference the standard variety RB867515. Measurements were made in plant cane during the winter season (August/September)

[c] The drought response of the genotypes evaluated was determined from IAC experimental network assays, in ratoon cane, as an index reflecting the ratio between the genotype performance under "cerrado" and normal rainfall conditions. Genotypes with a ratio > 0.9 were considered to be tolerant (data not shown)

Table 2 Sequence data of ten fixed primers and three arbitrary primers used to estimate the genetic variability in sugarcane genotypes

Primer	Sequences (5′→3′)	GenBank ID	Sequence ID	References
Fixed primer				
Susy	GGAGGAGCTGAGTGTTTC	AF263384	Sucrose synthase-2	Lingle and Dyer (2001) and Alwala et al. (2006)
SPS	CTACTTCGTCGAGGAGGT	AB001337	Sucrose-Phosphate Synthase	Sugiharto et al. (1997)
SAI	AGGACGAGACCACACTCT	AF062735	Soluble acid invertase	Alwala et al. (2006)
DirH	TGGAGATTTTTGGAGGAAC	TC48901	Dirigent protein	Calsa-Junior 2005)
DirL	CTTAACGAGGTGGTGGTGGT	TC57411	Putative dirigent protein	Calsa-Junior (2005)
Sut4	GATGGTGTGAGGATGGGTTC	TC69745	Sucrose transporter ZMSUT4	Calsa-Junior (2005)
Sut	GATTTGAATACCCTTGGAC	BU103671	Sugar transporter	Nogueira et al. (2003)
Aqua	ATCTCCGGCGGCCACAT	CA086489	Water channel	Vettore et al. (2001)
LEA	ATCTCCGGCGGCCACAT	BU103674	Late embryogenesis abundant protein	Vettore et al. (2001)
DBF	CTCTGCCACCACCACCTC	CA077947	Transcription factor	Vettore et al. (2001)
Arbitrary primer				
Arb1	GACTGCGTACGAATTAAT			Li and Quiros (2001)
Arb2	GACTGCGTACGAATTGAC			Li and Quiros (2001)
Arb3	GACTGCGTACGAATTTGA			Li and Quiros (2001)

values (r_{cof}) using the COPH program and then by comparing this matrix with the original distance matrix using the MXCOMP subprogram of NTSYS. The allelic diversity at a given locus was based on the polymorphism information content (PIC) measure (Satyavathi et al. 2006).

To quantify the existing genetic variability and understand how this variability was fractioned among the genotypes, we split the 46 Brazilian elite genotypes studied into three groups that represented SP, IAC–IACSP, and RB varieties, respectively. The analysis of molecular variance (AMOVA) was carried out using Arlequin ver. 3.01 (Excoffier et al. 2005) to detect variation within and among groups, and significance values were assigned to variance components based on the random permutation (1000 times) of individuals assuming no genetic structure. Dboot software (A. Coelho, personal communication), based on the bootstrap method (Efron 1981), was used to verify if the number of polymorphic TRAP markers used for GS estimation was adequate to provide precise estimates among the genotypes (Tivang et al. 1994). The polymorphic markers for sucrose metabolism and drought response were submitted independently to 1000 samplings one by one, with a growing replacement of markers. The average, the variance, and the coefficient of variation were estimated for each one of these combinations.

Results and discussion

Polymorphisms in sucrose metabolism and drought genes within a sample of 60 accessions of *Saccharum* spp. were evaluated using TRAP. For the sucrose metabolism candidate genes, the 21 primer combinations yielded 340 polymorphic bands, with a mean of 16.2 bands per primer combination. The largest number of fragments was observed with the SAI/Arb2 primer combination (30 fragments), and the lowest (ten fragments) with the SUSY/Arb2 and DirL/Arb3 primer combination. For the drought response candidate genes, the nine primer combinations resulted in 155 polymorphic fragments, with a mean of 17.2 fragments per primer combination. The largest number of polymorphic fragments was obtained with the Aqua/Arb2 primer combination (24 fragments), and the lowest with LEA/Arb2 (13 fragments). Alwala et al. (2006) found a mean of 29.38 polymorphic bands using 18 TRAP primer combinations on a set of 30 accessions that comprised hybrid accessions, one *Erianthus* accession, one *Miscanthus* accession, and 19 accessions of five *Saccharum* species. Despite the different genes evaluated in the two studies cited above, the different numbers of polymorphic bands must be due to differences in the genetic background of the accession group analyzed in both studies. The accession group analyzed by Alwala et al. (2006) was wider than the one in our study because it included a larger number of accessions from a larger number of species. On the other hand, the polymorphic loci found in our study were more informative, with the PIC ranging from 0.18 (DirL/Arb1) to 0.42 (SAI/Arb1), with an average of 0.30 (Table 3), than the ones found by Alwala et al. (2006).

Although a large number of polymorphic fragments (495 fragments) were identified for the whole sample, very few private alleles were observed in the cultivated genotypes analyzed. The absence of private alleles in the cultivated group may be due the fact that genotypes have common ancestors that are associated to strong selection pressure imposed under specific traits, such as sucrose content. The close relationship among the genotypes analyzed was also revealed in the AMOVA, which revealed that for either the drought or sucrose data, the majority of genetic variability was within the breeding program groups (97.89% for drought and 97.47% for sucrose) rather than among groups (2.11% for drought and 2.53% for sucrose) (Tables 4, 5).

Genetic similarity and cluster analysis

The grouping of varieties and species based on the polymorphism found in the two groups of genes analyzed are presented in Fig. 1. The dendrograms had a 0.96 cophenetic coefficient of correlation (r_{coph}) for the sucrose data and 0.91 for the drought data. In both dendrograms, a larger group was formed by clustering all of the sugarcane varieties with two accessions of *S. officinarum*: White transparent and *S. officinarum* 8272. *Saccharum spontaneum* (SES 205A), *S. robustum*, and the representative of genus *Erianthus* remained in separate clusters, which is agreement with the taxonomical classification proposed for "*Saccharum* complex" (Daniels et al.

Table 3 Primer combinations, number of fragments analyzed, and PIC values obtained with TRAP markers derived from candidate genes involved in sucrose metabolism and the drought response tolerance metabolism

Primer combination (fixed/arbitrary)	Number of fragments scored	PIC
Sucrose metabolism		
SuSy + Arb1	16	0.37
SuSy + Arb2	10	0.36
SuSy + Arb3	17	0.32
SPS + Arb1	13	0.35
SPS + Arb2	16	0.30
SPS + Arb3	16	0.28
SAI + Arb1	22	0.42
SAI + Arb2	30	0.33
SAI + Arb3	18	0.35
SUT4 + Arb1	17	0.39
SUT4 + Arb2	17	0.33
SUT4 + Arb3	11	0.29
DirH + Arb1	17	0.28
DirH + Arb2	14	0.21
DirH + Arb3	21	0.24
DirL + Arb1	13	0.18
DirL + Arb2	13	0.28
DirL + Arb3	10	0.21
SUT + Arb1	14	0.37
SUT + Arb2	16	0.26
SUT + Arb3	19	0.26
Total	340	–
Average	16.2	–
Drought response		
Aqua + Arb1	16	0.35
Aqua + Arb2	24	0.19
Aqua + Arb3	20	0.35
DBF + Arb1	17	0.32
DBF + Arb2	15	0.37
DBF + Arb3	15	0.32
LEA + Arb1	15	0.32
LEA + Arb2	13	0.25
LEA + Arb3	20	0.29
Total	155	
Average	17.2	0.30

1975). One explanation of the clustering of the sugarcane varieties with *S. officinarum* is based on the ancestral origin of modern varieties from *S. officinarum*. White transparent constituted a very important genotype during the nobilization process and is still used today as a parent for generating new genotypes in Brazilian breeding programs. The close relationship among varieties and *S. officinarum* was also observed by Alwala et al. (2006) using TRAP markers derived from candidate genes for sucrose and cold tolerance metabolism.

The accessions were grouped in several clusters based on both data sets. Some of these clusters comprised the same accessions for both data sets. For example, varieties IAC91-4168, IAC91-2205, IAC91-2195, and IAC91-2218 were grouped together based on sucrose and drought data. However, the relationships between these clusters in general varied according the data set. On the other hand, some clusters were exclusive for only one of the dendrograms.

Some varieties that have common parents were grouped together (varieties RB855156 and RB855595; IAC86-3154 and IAC86-2210) based on sucrose and drought data. For the dendrogram generated from sucrose data, a subgroup included three RB varieties (RB72454, RB855453 and RB855156), which were also grouped into a cluster using AFLP markers in sugarcane (Lima et al. 2002).

Clusters including varieties from the three breeding programs (IAC–IACSP, SP, and RB) were not evident. Such varieties are descended from common ancestors and have been intensively explored by the three breeding programs. Examples of such varieties are NA56-79, SP79-1011, RB72454, and SP70-1143.

The clusters included genotypes with different sugar content (Pols). Sugar content is a trait controlled by several genes, and it is likely that some extra genes involved in sucrose metabolism need to be included in the analysis. Also, some of the polymorphisms detected may be in gene regions that do not account for phenotypic differences, or the polymorphisms between alleles that account for phenotypic differences between accessions may not be detected since they are due to point mutations, which are not reflected in the length. Another possible explanation is the close proximity of the ranges used to define rich (>15% Pol) and poor (12–15% Pol) accessions, making it difficult to separate genotypes.

The IAC87-3396, SP70-1143, and RB83-5486 varieties were clustered together on the dendrogram obtained for drought data. These varieties have

Table 4 Analysis of molecular variance among sugarcane varieties from the three different Brazilian sugarcane breeding programs based on TRAP markers derived from drought response metabolism genes

Source of variation	*df*	Sum of squares	Variance components	Percentage of total variation
Among groups	2	57.893	0.485	2.11
Within groups	43	967.346	22.496	97.89
Total	45	1025.239	22.982	

Table 5 Analysis of molecular variance among sugarcane varieties from the three different Brazilian sugarcane breeding program based on TRAP markers derived from sucrose metabolism genes

Source of variation	*df*	Sum of squares	Variance components	Percentage of total variation
Among groups	2	129.921	1.252	2.53
Within groups	43	2078.231	48.331	97.47
Total	45	2208.152	49.582	

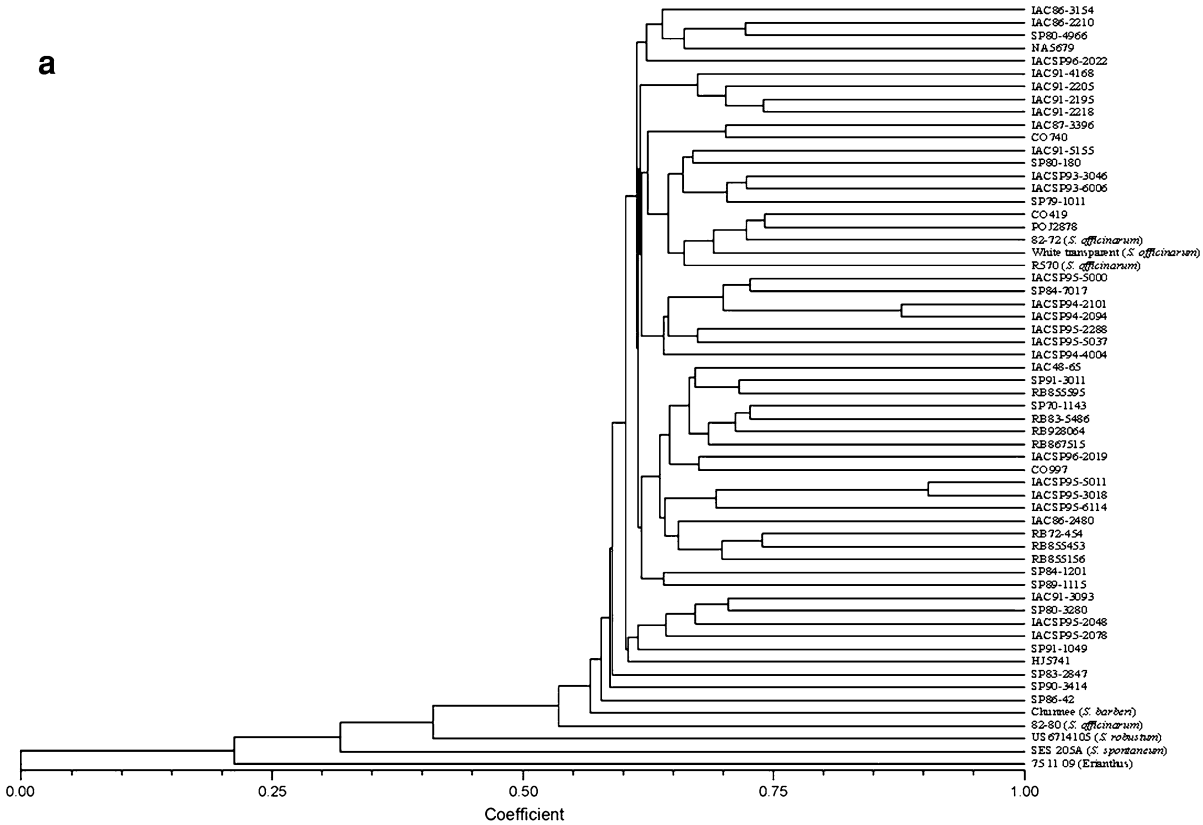
shown good performance in Brazilian "cerrado" (savanna) areas and, based on field observations, can be considered to be tolerant to drought. The clustering of these genotypes together based on the common alleles that they share may be due to a common drought response mechanism, which may in turn be derived from the same source, since many of the genotypes have common ancestors. Lima et al. (2002) characterized these three varieties using amplified fragment length polymorphism (AFLP) markers and found that they were not closely related, as they clustered in separate groups in the dendrogram. It is accepted that crosses between unrelated genotypes will maximize the number of segregating alleles, resulting in a large genetic variance in the progeny (Cox et al. 1985; Messmer et al. 1993), thereby increasing the opportunity for selecting rare genotypes that may be superior (Becelaere et al. 2005). Thus, GD must be estimated based on different data sources. For example, if crossings between the varieties listed above were established on the basis of only random markers, this could lead breeders to select genotypes carrying the same alleles for a specific trait. TRAP markers are derived from candidate genes representing functional markers that may be directly involved with a phenotypic trait variation. Moreover, in our study, specific regions of the sugarcane genome related to drought tolerance, rather than the entire genome, were sampled to evaluate the genetic variability of the important parents of the Brazilian sugarcane breeding programs. In our study, the lowest similarity value for drought was obtained between the genotypes IACSP95-2078 and SP86-42 (0.44), illustrating that this cross would probably result in the highest variability for drought among the genotypes sampled. The SP86-42 variety has excellent performance in the Brazilian "cerrado" areas and has been grown in drought-prone environments.

Based on the sucrose metabolism gene data, the lowest GS value was obtained between the IACSP94-2101 and IAC86-3154 genotypes (0.52). Thus, the highest segregation for the sucrose genes evaluated would be achieved if these two accessions were crossed. However, IACSP94-2101 flowers under exceptional natural conditions and, therefore, it is not commonly used as a parent in sugarcane crosses.

Although the dendrograms obtained for both traits did not show important differences in the clustering of the genotypes, the GD between genotypes were different when based on drought or sucrose metabolism data. The range of variation in GS values was smaller for the sucrose (0.52–0.90) than for drought (0.44–0.95) values (Fig. 2).

It is interesting to note that the lower GS value was higher (0.52) for the sucrose data than for the drought (0.44) data, possible due to the fact that sucrose

Fig. 1 Dendrogram revealed by unweighted pair-grouping ▶ with arithmetic average cluster analysis of TRAP-based genetic similarity (Jaccard's coefficient) estimates from sucrose (**a**) and drought (**b**) metabolism genes


a
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IAC86-2210
SP80-4966
NA5679
IACSP96-2022
IAC91-4168
IAC91-2205
IAC91-2195
IAC91-2218
IAC87-3396
CO740
IAC91-5155
SP80-180
IACSP93-3046
IACSP93-6006
SP79-1011
CO419
POJ2878
82-72 (S. officinarum)
White transparent (S. officinarum)
R570 (S. officinarum)
IACSP95-5000
SP84-7017
IACSP94-2101
IACSP94-2094
IACSP95-2288
IACSP95-5037
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SP86-42
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82-80 (S. officinarum)
US 6714105 (S. robustum)
SES 205A (S. spontaneum)
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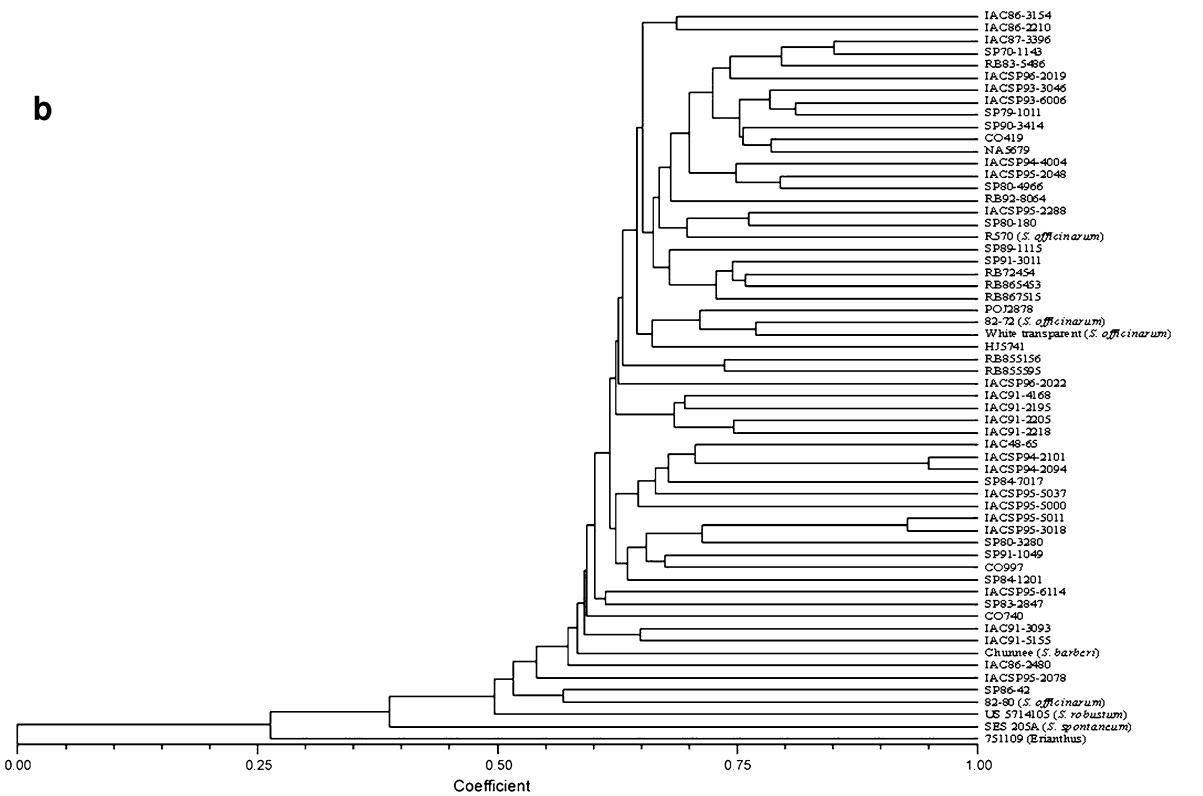
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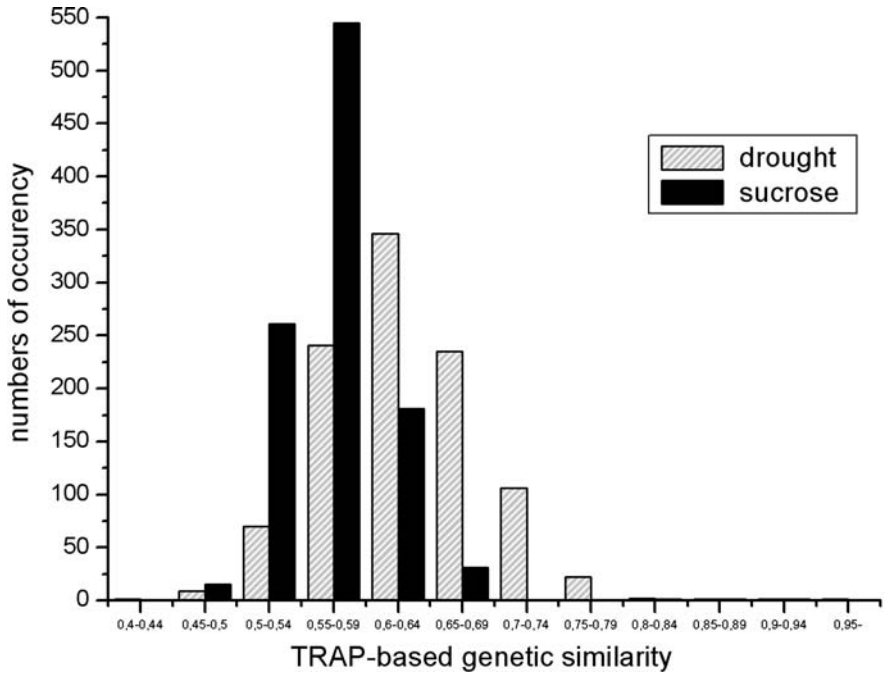

Fig. 2 Frequency distribution of TRAP-based GS values for 1035 pairs of sugarcane elite parents

content is the major focus of sugarcane breeding programs and, as such, it has been under higher selection pressure than other traits. It is a well-known among sugarcane breeders that the small number of initial clones from which modern sugarcane varieties are derived have been subjected to an intensive selection for sucrose content, thereby limiting the gain in sugar content (Aitken et al. 2006; Jackson 2005; Grof et al. 2007). On the other hand, drought tolerance has only recently received attention in Brazilian sugarcane breeding programs, due to the expansion of the sugarcane crop to the "cerrado" areas. Therefore, there is a higher genetic variability among the genotypes for drought and, as a consequence, it is expected that the potential for progress in breeding programs is greater. Indeed, this fact has been noted in field experiments by our team of breeders.

Using all the 340 polymorphic bands from sucrose metabolism and the 155 polymorphic bands from drought metabolism, the coefficient of variance (CV) values reflecting experimental error were 5.5 and 7.7 respectively. These values are seen to be reliable for appropriate GS estimation, since some authors recommend a 10% CV (Lima et al. 2002). Alwala et al. (2006) obtained an 8.1% CV using 242 TRAP bands in nine sugarcane parents. Lima et al. (2002), working with AFLPs markers in sugarcane, obtained a 4.29% CV value using 1121 polymorphic bands. Hence, it is reasonable to infer that the TRAP markers presented in our study were suitable for evaluating the GD accessed at the function level, i.e., sucrose and drought metabolism genes in sugarcane germplasm.

Our data suggest that some of the difficulties facing breeders in their drive to improve sucrose content are due to the narrow gene pool used in current commercial breeding program. They also suggest that those gains which have been obtained in generating new varieties are most likely related to G × E interaction due to the correct allocation of varieties in a specific environment—and not necessarily to an overall genetic gain per se.

The identification of new alleles for sucrose content in *S. officinarum* or *S. spontaneum* accessions associated to an introgression breeding program with sugarcane elite materials should contribute to improved gains in sugar content and productivity, as demonstrated recently by Grof et al. (2007) and Wang et al. (2008).

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