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Genetic diversity analysis of *Saccharum spontaneum* germplasm using SSR-SSCP and RAPD markers

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ABSTRACT

Saccharum spontaneum, the most important wild species used in the evolution of modern sugarcane varieties, imparts vigour, cold hardiness and tolerance towards biotic and abiotic stresses. Fourteen genotypes of *S. spontaneum* genotypes being maintained at Indian Institute of Sugarcane Research, Lucknow, were subjected to genetic diversity analysis using microsatellites and randomly amplified polymorphic DNA (RAPD) markers. Nine sugarcane specific flanking sequences of di and trinucleotide SSR (simple sequence repeat) motifs of EST (expressed sequence tags) and genomic DNA (Deoxyribonucleic acid) origin and 68 random operons were used for amplification. Single strand conformational polymorphism (SSCP) analysis of SSR amplicons yielded 128 conformers and, through RAPD 961 bands were amplified. Use of two types of markers amplifying different regions of the genome, allowed better analysis of genetic diversity; extensive molecular variability corroborated by the wide range of Dice similarity coefficients (0.04 to 0.48 in SSR-SSCP and 0.09 to 0.60 in RAPD) was observed. UPGMA (unweighed pair group method of arithmetic average) of similarity coefficients separated the genotypes in two main clusters. Identification of genetically distant genotypes of *S. spontaneum* would augment sugarcane breeding through the use of diverse parents facilitating the selection of hybrids with maximum genetic diversity and hybrid vigour.

Key words: Genetic similarity, Germplasm, Polymorphism, RAPD, Sugarcane

Modern commercial sugarcane varieties (*Saccharum* spp hybrids, 2*n* =100–130) are derived from interspecific hybridization pioneered in Java (Sreenivasan *et al*. 1987). Before that, the improvement of sugarcane relied on the selection of naturally occurring variants of noble cane *S. officinarum.* The sugarcane breeding programme underwent radical changes with the utilization of wild *S. spontaneum* in the late eighteenth century and the early nineteenth century for incorporating the much sought disease resistance into cultivars, which marked the end of the noble cane era. The importance of the wild species *S. spontaneum* was realized after its successful hybridization with the cultivated species, *S. officinarum*, resulting in the production of first commercial hybrids Co 205 and Co 285, which replaced the indigenous cultivated varieties of northern India (Sreenivasan *et al*. 1987). Even after 10 decades of sugarcane breeding work, the utilization of *S. spontaneum* has remained limited. While in the world collection maintained by Sugarcane Breeding Institute, Coimbatore, there are more than 200 clones of *S. spontaneum*, not many are effectively used. It is obvious that there is a large reserve of untapped genetic potential of *S. spontaneum* (Roach 1971). A collection of *S. spontaneum* genotypes is being maintained at Indian Institute of Sugarcane Research (IISR), Lucknow under germplasm enhancement programme. The assessment of genetic diversity among these genotypes through DNA-based markers would aid breeding programmes aimed at widening of genetic base of sugarcane. Among the DNA-based markers, microsatellites, also known as simple sequence repeats (SSRs) are the ideal ones because of their abundance, high polymorphism and transferability across genotypes within a species. Microsatellites have already shown their potential as genetic markers in sugarcane (Cordeiro *et al*. 2000, D'Hont and Glaszmann 2001, McIntyre *et al.* 2001, Piperidis *et al*. 2001). Application of SSCP (single-strand conformation polymorphism) to SSR amplified products helps detect conformational changes in single stranded DNA molecules and hence enhances polymorphism of microsatellite repeats to facilitate molecular genetic diversity analysis (Srivastava *et al.* 2005). SSCP is based on electrophoretic detection of conformational changes in single stranded DNA molecules resulting from point mutations or other forms of small nucleotide changes. It was originally

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developed for rapid analysis of mutations (Orita *et al*. 1989). RAPD (randomly amplified polymorphic DNA) analysis employs single short primers with arbitrary sequences to generate genome-specific fingerprints of multiple amplification products (Welsh and McClelland 1990, Williams *et al*. 1990). Several studies have used RAPDs to assess levels and patterns of variation among various sugarcane varieties, species as well as members of *Saccharum* complex and to identify putative markers linked to phenotypic traits (Harvey and Botha 1996, Nair *et al*. 1999, 2002). In the present study, potential use of these markers was investigated in an attempt to characterize the molecular diversity of fourteen *S. spontaneum* genotypes (BG 01, BG 06, BG 09, BG 14, BG 20, BG 23, BG 24, Bazpur 2, Bazpur 7, SES 65, SES 72, SES 32 A, SES 597 and Gomti Mohamadi) available in the germplasm collection at IISR, Lucknow.

MATERIALS AND METHODS

Approximately 2 g of young leaf tissue of each genotype was used to extract and purify genomic DNA (Srivastava and Gupta 2001). The DNA was quantified in 0.8% (w/v) agarose gels by comparison with known quantities of the lambda phage DNA and stored at –20°C. Nine microsatellite primer pairs (derived from expressed sequence tag libraries and genomic DNA libraries) comprising dinucleotide and trinucleotide SSR motifs (Table 1) were used as flanking primers to amplify the gDNA extracted from leaves of *Saccharum spontaneum* genotypes. Amplification reactions were carried out in a PTC 200 in medium containing 1 X PCR buffer, 1.25mM d NTPs, 2 µM primer reverse and forward each, 4mM $MgCl_2$, 1 Unit Taq polymerase and 10 ng template DNA. Amplified products were denatured, snap chilled to convert them in single strands, separated on 10% (m/v) PAGE using TBE buffer and stained with ethidium bromide. Sixty eight random decamers taken from different Operon kits were used for RAPD-PCR (Table 2). Cycling was performed as 45 cycles of 94°C for 1 min, 37°C for 1 min and 72° C for 2 min with initial denaturation cycle of 94 ° C for 5 min and a final primer extension cycle of 72°C. The DNA polymerase was purchased. Each amplification experiment was performed at least twice. 2 µl of amplified products were run on 2% agarose gels in 1X TAE buffer in BioRad SubCell GT electrophoresis unit and stained by 0.5 µg/ml of ethidium bromide (EtBr) to determine DNA fragment products. Electrophoresed samples were photographed using AlphaImager^{TM} Gel documentation system. Data on reproducible polymorphic bands were scored for the presence or absence in each sample. Positions of unequivocally scorable bands were transformed into a binary character matrix (1 for the presence and 0 for the absence of a band at a particular position), which was used to estimate genetic similarity matrix through Dice similarity coefficient. The genetic symmetry matrices generated from the SSR-SSCP and RAPD markers were used to perform cluster analysis using the UPGMA (unweighed pair group method with arithmetic mean clustering) method (Sneath and Sokal 1973) following the SAHN (sequential agglomerative hierarchical nested) cluster analysis module. UPGMA the most straightforward method for tree construction, uses a sequential agglomerative clustering algorithm where individuals, technically referred

Table 1 Microsatellite primers used in the study, their sequences, source, number of alleles and product size range of conformers in *S. spontaneum* genotypes

Primer	Nucleotide sequence	SSR motif	Source/origin	No. of markers	Product size range (bp)	
					Expected	Observed
NKS 1	5'-TGGCATGTGTCATAGCCAAT-3' 5'-CCCCAACTGGGACTTTTACA-3'	(GAA)6	EST	12	207–228	172–250
NKS 2	5'-GCTGTCCCGTTCCAAGTTAC-3' 5'-GCGACCGGATTATGATGATT-3'	(GA)13	EST	6	185–216	139–323
NKS 17	5'- GCTCGCCATGAATAGAAAGG-3' 5'- ACCGAGGTAGGAGGGAGTGT-3'	(GA)24	Genomic	19	206–227	130 – 268
mSSCIR 5	5'-GCAGCCTTGGTTCGGTCTATG-3' 5'-GCATCCCTCGCCCTTCCTC-3'	(GGC)9	Genomic	17	379	233–555
mSSCIR 20	5'-CTTTTGTGGGATGTTGAT-3' 5'-CTCATTTACAACCAGGAT-3'	(GT)7	Genomic	6	197	116–231
mSSCIR 25	5'-TTGCCGTTGCCTGCTCT-3' 5'-CACGCACTCCACTCACACC-3'	(GA)24	Genomic	9	300	191–403
mSSCIR 28	5'-CCGCATCTCTTTGTTTTG-3' 5'-GGTGGTGATGAGTCGTGA-3'	(GA)34	Genomic	16	419	257–539
mSSCIR 61	5'-CCCCATTTCTCCGTTACCG-3' 5'-CCACCACCAACCTCATCTCC-3'	(GT)27	Genomic	29	200–300	209–476
mSSCIR 76	5'-GCGAACCAAGGAGAAGCA-3' 5'-TCCACCGAGTTCCCATTG-3'	(GGC)8	Genomic	14	325	276–478

Table 2 Random operon primers used in the study, their sequences, number of markers resolved and their product size range in *S. spontaneum* genotypes

Operon primers	Sequences (5'-3')	Product size (bp)	No. of RAPD markers	Operon primers	Sequences (5'-3')	Product size (bp)	No. of RAPD markers
OPA 01	5'-CAGGCCCTTC-3'	356-2231	17	OPJ 20	5'-AAGCGGCCTC-3'	478-1247	11
OPA 02	5'-TGCCGAGCTG-3'	265-2622	12	OPK 07	5'-AGCGAGCAAG-3'	274-1242	16
OPA 04	5'-AATCGGGCTG-3'	391-1910	17	OPK 12	5'-TGGCCCTCAC-3'	395-1585	18
OPA 07	5'-GAAACGGGTG-3'	475-2729	15	OPAA 01	5'-AGACGGCTCC-3'	397-1493	9
OPA 08	5'-GTGACGTAGG-3'	559-2457	8	OPAA 04	5'-AGGACTGCTC-3'	251-1884	18
OPA 10	5'-GTGATCGCAG-3'	314-1986	17	OPAA 07	5'-CTACGCTCAC-3'	318-1131	13
OPA 12	5'-TCGGCGATAG-3'	317-850	7	OPAA 16	5'-GGAACCCACA-3'	351-1366	14
OPA 16	5'-AGCCAGCGAA-3'	758-2255	12	OPAA 12	5'-GGACCTCTTG-3'	318-1237	9
OPA 17	5'-GACCGCTTGT-3'	641-1978	11	OPAA 18	5'-TGGTCCAGCC-3'	380-1313	8
OPA 19	5'-CAAACGTCGG-3'	273-1851	18	OPAA 20	5'-TTGCCTTCGG-3'	451-2149	20
OPB 01	5'-GTTTCGCTCC-3'	623-1225	18	OPAA 02	5'-GAGACCAGAC-3'	806-1241	5
OPB 04	5'-GGACTGGAGT-3'	363-2773	9	OPAB 01	5'-CCGTCGGTAG-3'	280-1158	13
OPB 05	5'-TGCGCCCTTC-3'	824-2407	5	OPAB 04	5'-GGCACGCGTT-3'	253-1263	16
OPB 06	5'-TGCTCTGCCC-3'	714-2065	10	OPAB 05	5'-CCCGAAGCGA-3'	242-2081	17
OPB 07	5'-GGTGACGCAG-3'	461-1847	16	OPAB 07	5'-GTAAACCGCC-3'	368-1065	10
OPB 18	5'-CCACAGCAGT-3'	527-2407	14	OPAK 01	5'-TCTGCTACGG-3'	427-2579	15
OPB 08	5'-GTCCACACGG-3'	721-2499	10	OPAK 03	5'-GGTCCTACCA-3'	420-2479	12
OPB 09	5'-TGGGGGACTC-3	351-2286	14	OPAK 04	5'-AGGGTCGGTC-3'	349-2111	29
OPB 10	5'-CTGCTGGGAC-3'	318-2346	9	OPAK 05	5'-GATGGCAGTC-3'	1112-1723	3
OPB 11	5'-GTAGACCCGT-3'	332-1875	16	OPAK 06	5'-TCACGTCCCT-3'	469-2410	22
OPB 15	5'-GGAGGGTGTT-3'	506-2496	13	OPAK 07	5'-CTTGGGGGAC-3'	322-2012	29
OPC 07	5'-GTCCCGACGA-3'	677-1876	11	OPAK 08	5'-CCGAAGGGTG-3'	425-2310	18
OPC 04	5'-CCGCATCTAC-3'	373-1626	11	OPAK 09	5'-AGGTCGGCGT-3'	297-1927	21
OPC 16	5'-CACACTCCAG-3'	487-2010	13	OPAK 10	5'-CAAGCGTCAC-3'	444-1963	16
OPD 06	5'-ACCTGAACGG-3'	298-1884	15	OPAK 11	5'-CAGTGTGCTC-3'	613-1989	13
OPD 19	5'-CTGGGGACTT-3'	486-2360	12	OPAK 12	5'-AGTGTAGCCC-3'	608-1943	11
OPE 08	5'-TCACCACGGT-3'	326-1275	12	OPAK 13	5'-TCCCACGAGT-3'	348-2074	15
OPE 06	5'-AAGACCCCTC-3'	270-1922	19	OPAK 14	5'-CTGTCATGCC-3'	810-2350	16
OPF 14	5'-TGCTGCAGGT-3'	411-1370	13	OPAK 15	5'-ACCTGCCGCT-3'	311-1904	10
OPG 06	5'-GTGCCTAACC-3'	347-1562	17	OPAK 16	5'-CTGCGTGCTC-3'	321-1811	10
OPH 09	5'-TGTAGCTGGG-3'	239-1194	10	OPAK 17	5'-CAGCGGTCAC-3'	414-2098	24
OPI 06	5'-AAGGCGGCAG-3'	398-1133	9	OPAK 18	5'-ACCCGGAAAC-3'	313-2341	24
OPI 19	5'-AATGCGGGAG-3'	419-1678	13	OPAK 19	5'-TCGCAGCGAG-3'	459-2009	19
H 6	5'-ACCGAAGCCC-3'	346-1417	16	OPAK 20	5'-TGATGGCGTC-3'	312-1692	18

to as operational taxonomic units (OTUs) (Weir 1996), are clustered in order of similarity, and the tree is built in a step-wise manner. The goodness-of-fit of the dendrograms to the original genetic symmetry/dissymmetry matrix was calculated by computing the co-phenetic value (rcoph) using the COPH (cophenetic) and MXCOMP (matrix comparison) modules. The COPH module computes a symmetrical matrix of cophenetic (ultrametric) similarity or dissimilarity values in the form of a tree matrix from the set of nested clusters produced by the SAHN clustering module. The MXCOMP module tests the goodness of fit of the cluster analysis and the original GS matrix by computing the product-moment correlation, r, and the Mantel test statistic, Z, to measure the degree of relationship between the two matrices. A rcoph = 80% is generally considered a good fit (Rohlf 2000). All statistical analysis was done by using the software NTSYSpc version 2.11V (Rohlf 2000).

RESULTS AND DISCUSSION

Electrophoretic analyses of SSCP on 10% PAGE using SSR primers provided reliable distinct multiple band profiles for fourteen *S. spontaneum* genotypes (Fig 1a). A total of 215 conformers were analyzed in these genotypes, which belonged to 128 bands of distinct molecular weight having approximately 99% polymorphism. An average of 15.35 fragments per genotype were amplified. Total number of

bands amplified for each primer ranged from 6–29 (Table 1), with an average of 14.22 fragments/primer. Eight primers showed complete parsimony and were very useful for diversity purpose.

Using 68 primers taken from Operon kits A, B, C, D, E, F, G, H, I, J, K, AA, AB and AK to amplify genomic DNA from fourteen *S. spontaneum* genotypes, distinct multiple band profiles were obtained (Fig 1b). A total of 3459 RAPD bands were produced across all the genotypes with 3 to 29 amplicons/primer in different genotypes (Table 2). Among these, approximately 99% bands were polymorphic. An average of 247.07 fragments/genotypes and 50.86 fragments/primer were amplified. The molecular weight of PCR amplification products ranged from 239 to 2 773 bp (Table 2). The bands having same molecular weight were considered as one RAPD marker; thus a total of 961 RAPD markers were resolved.

The data from SSR-SSCP and RAPD was subjected to Dice similarity coefficient analysis. The similarity coefficients for SSR markers ranged from 0.04 (BG 14 and Bazpur 2, Bazpur 7 and SES 72 and Bazpur 7 and Gomati Mohamadi) to 0.48 (BG 6 and BG 24) with a mean similarity coefficient of 0.20 (Table 3). The Dice similarity coefficients for RAPD ranged from 0.09 (BG 23 and BG 24) to 0.60 (BG 6 and BG 20) (Table 4) with a mean similarity value of 0.34 suggesting that these *S. spontaneum* genotypes were quite diverse.

Molecular diversity in *S. spontaneum* genotypes using 128 SSCP conformers generated by 9 SSR primers and 961 RAPD markers resolved through 68 random primers became apparently more clear through the dendrograms (Fig 2a b) generated by UPGMA (unweighted pair group method with arithmetic mean clustering)-based cluster analysis performed on the matrix of DICE coefficients of genetic similarity. Two taxonomical groups were clearly resolved with both the marker systems. The first taxonomical group consisted of

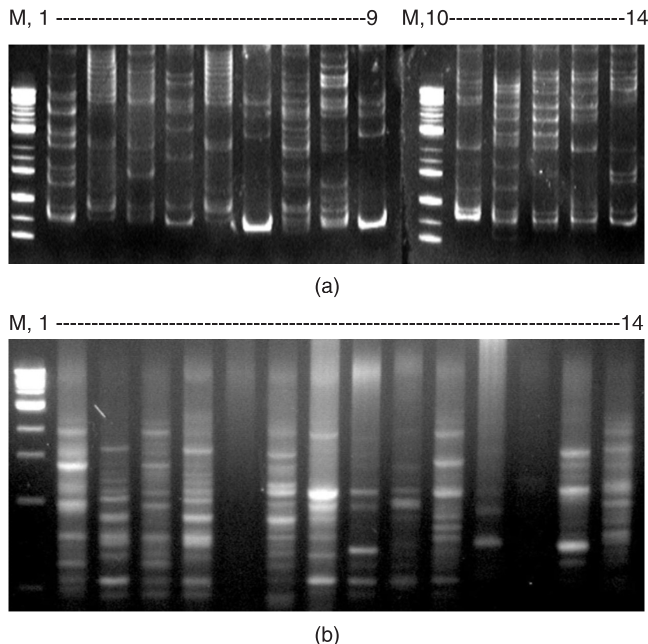

Fig 1 PCR amplification profile of *S. spontaneum* genotypes (*a*) SSR-SSCP profile with primer pair NKS 1, and (*b*) RAPD profile using primer OPAK 19

M 100 bp DNA ladder; Lane 1 to 14 M BG 01, BG 06, BG 14, BG 20, Bazpur-2, BG 23, SES 65, SES 72, SES 32A, M, SES 597, Bazpur 7, BG 24, Gomti Mohamadi and BG 09

clones belonging to 12 genotypes consisting of BG types, SES types, and Gomati Mohamadi. The other taxonomical group clubbed the clones of Bazpur type. The dendrograms obtained with microsatellites and RAPD markers agreed in principle, in clustering the genotypes assayed. The BG and SES types along with Gomti Mohamadi clustered together at X-axis with further subclusters. Among these, all BG types as well as SES types were grouped together in one subcluster

Table 3 DICE similarity coefficient matrix of *S. spontaneum* genotypes based on nine microsatellite primers

Genotype	BG 01	BG 06	BG 14	BG 20	BG 23	Bazpur 2	SES 65	SES 72	SES 32A	SES 597	Bazpur 7	BG 24	Gomti Mohamadi	BG 9
BG 01	1.00													
BG 06	0.40	1.00												
BG 14	0.34	0.13	1.00											
BG 20	0.43	0.34	0.36	1.00										
BG 23	0.17	0.16	0.21	0.21	1.00									
Bazpur 2	0.21	0.27	0.06	0.18	0.14	1.00								
SES 65	0.19	0.13	0.17	0.29	0.13	0.11	1.00							
SES 72	0.25	0.18	0.16	0.17	0.13	0.22	0.26	1.00						
SES 32A	0.24	0.31	0.27	0.21	0.16	0.13	0.44	0.24	1.00					
SES597	0.24	0.15	0.20	0.14	0.16	0.20	0.25	0.30	0.23	1.00				
Bazpur 7	0.08	0.15	0.13	0.07	0.16	0.33	0.13	0.06	0.08	0.08	1.00			
BG 24	0.29	0.48	0.12	0.31	0.29	0.36	0.23	0.22	0.21	0.28	0.21	1.00		
Gomti Mohamadi	0.20	0.13	0.11	0.12	0.13	0.17	0.16	0.21	0.19	0.13	0.06	0.18	1.00	
BG 09	0.29	0.41	0.18	0.31	0.14	0.18	0.17	0.17	0.21	0.28	0.07	0.25	0.12	1.0

Table 4 DICE similarity coefficient matrix of *S. spontaneum* genotypes based on 68 RAPD primers

Genotype	BG 01	BG 06	BG 14	BG 20	BG 23	Bazpur 2	SES 65	SES 72	SES 32A	SES 597	Bazpur 7	BG 24	Gomti Mohamadi	BG 9
BG 01	1.00													
BG 06	0.46	1.00												
BG 14	0.48	0.46	1.00											
BG 20	0.41	0.60	0.41	1.00										
BG 23	0.14	0.15	0.11	0.15	1.00									
Bazpur 2	0.41	0.51	0.42	0.49	0.19	1.00								
SES 65	0.39	0.43	0.42	0.40	0.17	0.49	1.00							
SES 72	0.41	0.48	0.43	0.40	0.2	0.49	0.48	1.00						
SES 32A	0.37	0.43	0.39	0.52	0.12	0.52	0.45	0.48	1.00					
SES597	0.41	0.45	0.49	0.40	0.12	0.45	0.45	0.46	0.44	1.00				
Bazpur 7	0.14	0.15	0.11	0.15	0.36	0.20	0.15	0.18	0.16	0.15	1.00			
BG 24	0.41	0.44	0.44	0.43	0.09	0.45	0.44	0.46	0.44	0.52	0.13	1.00		
Gomti Mohamadi	0.29	0.26	0.29	0.30	0.13	0.32	0.27	0.36	0.33	0.30	0.15	0.32	1.00	
BG 09	0.35	0.36	0.33	0.50	0.11	0.39	0.31	0.34	0.47	0.34	0.15	0.37	0.31	1.0

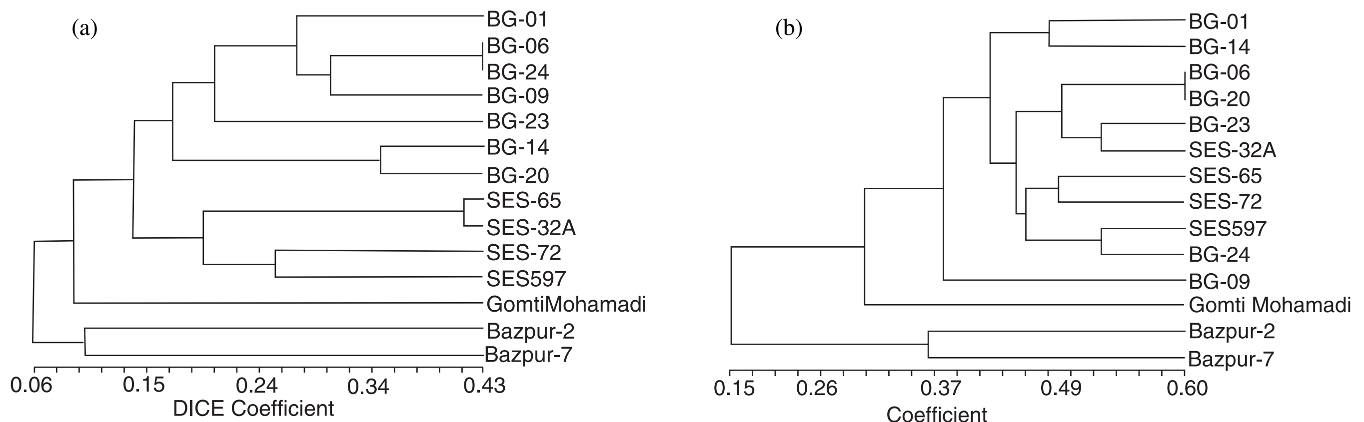

Fig 2 UPGMA-based dendrograms indicating genetic relationship of *S. spontaneum* genotypes, (*a*) based on SSR-SSCP markers and (*b*) based on RAPD markers

in both the dendrograms. Similarly, Bazpur 2 and Bazpur 7 were clustered together at Y-axis in both the dendrograms. To measure the goodness of fit for the cluster analysis a cophenetic correlation (r_{coph}) of the cophenetic (ultrametric) value matrix (Rohlf and Sokal 1981) and the matrix upon which the clustering was based, was computed. Comparison of the cophenetic values obtained from the UPGMA cluster analysis, with symmetry matrix for RAPD demonstrated a correlation of 0.97 indicating that data in the matrix was represented very well by the dendrogram. However, for SSCP-SSR, the goodness of fit test for the cluster analysis resulted in a slightly lower correlation (r =0.76), indicating fairly well defined clusters.

To provide an objective comparison, matrices of cophenetic values, generated from RAPD. The correlation coefficient of RAPD and microsatellites matrices using 2-way Mantel test after doing 250 random permutations was r= 0.46. Probably correlation between them could be improved if there were more number of SSR markers analyzed. The results of dendrogram analysis were corroborated by means of multi-dimensional scaling (MDS) analysis (Kruscal 1964), an ordination technique that reveals patterns of relatedness within a matrix by assigning Cartesian coordinates in a multi-dimensional space to each genotype. MDS compliments cluster analysis by providing spatial representation of relative genetic distances among genotypes (Fig 3). The two dimensional MDS using 961 RAPD markers provided an excellent compromise between fit to the genetic diversity estimate matrix (stress 2= 0.02154) and visual interpretation (Fig 3b) confirming the genetic relationship obtained in the dendrogram analysis. However the two dimensional MDS using 128 SSR markers provided only a fair fit of genetic diversity estimate matrix (stress 2= 0.2989) and visual interpretation (Fig 3a). This reinforces, as suggested before, that more number of SSR markers should be analyzed.

Several workers have used random amplified

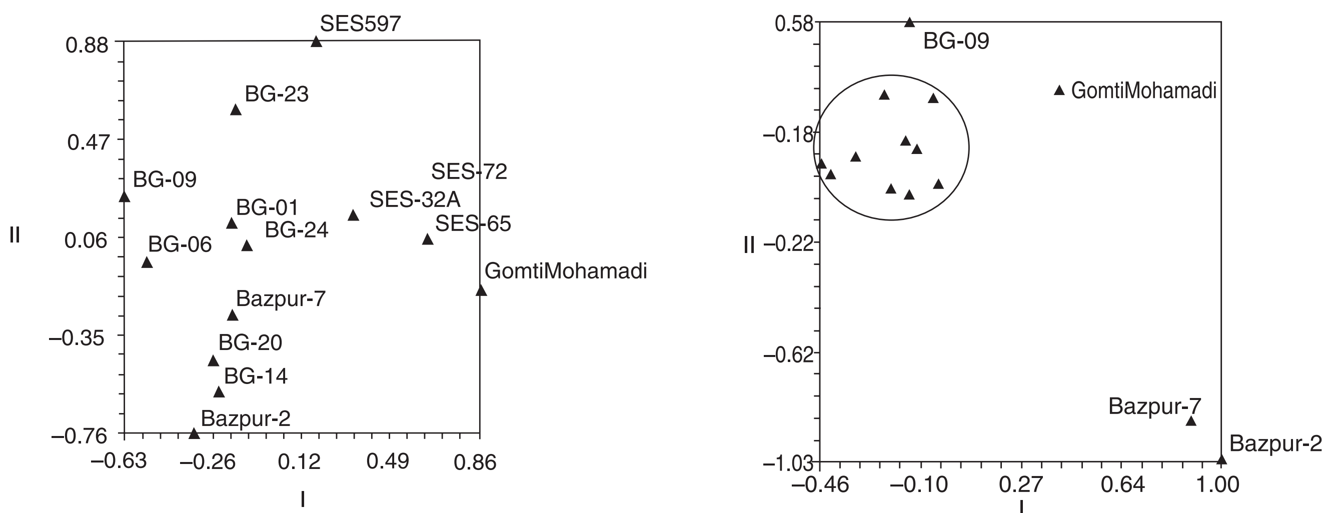

Fig 3 Multidimensional scaling of *S. spontaneum* genotypes. Diversity estimates are based on (*a*) 128 SSCP-SSR markers and (*b*) 961 RAPD markers. The circle in (*b*) contains genotypes BG 01, BG 06, BG 14, BG 20, BG 23, BG 24, SES 65, SES 72, SES 32A and SES 597

polymorphic DNA (RAPD) markers to identify sugarcane genotypes. Oropeza and Garcia (1997) used RAPD analysis to detect polymorphism in *Saccharum officinarum* lines PR62258 and V781. Fernández *et al.* (1999) evaluated genomic diversity of 40 commercial sugarcane varieties of Cuba including four somaclones and the donor (C8751). Ubayasena (1999) screened out nine polymorphic primers after a pre-screening of sixty primers using two bulk DNA samples representing *S. spontaneum* and *Erianthus* and found that the genetic diversity of the investigated accessions ranged from 0% to 69.23%. RAPD analysis using sixty eight primers in the present study differentiated *S. spontaneum* genotypes efficiently. The level of polymorphisms among the *S. spontaneum* genotypes tested indicates that distinction between any two genotypes should be possible with appropriate primers. Nei (1978) suggested that a minimum number of 50 different loci should be used for estimating genetic distances. Fernández *et al.* (1999) used 18 random primers and detected 77 polymorphic bands from a total of 106 bands in sugarcane varieties of Cuba. Use of 961 RAPD markers exhibiting 99% polymorphism among the genotypes in the present study to determine the genetic relatedness among 14 genotypes of *S. spontaneum* was adequate. An average number of 14.07 polymorphic bands/primer were obtained in this study. Comparative values in some other plants range from 3.8 polymorphic bands/primer in rapeseed (Mailer *et al.,* 1994), 3.9 in rice (Song *et al.* 1992), 5.6 in sugarcane (Fernández *et al.* 1999), 6.0 in *Hordeum* (Volis *et al*. 2001), 11.3 in *Lansium domesticum* (Song *et al.* 2000), 12.9 in *Lathyrus* (Chtourou-Ghorbel *et al.* 2001), 15.3 in Piper (Pradeep Kumar *et al.* 2001), and 40.8 in *Orobanche* (Roman *et al.* 2003).

Comparison of dendrograms indicating genetic relatedness among fourteen *S. spontaneum* genotypes based on RAPD and SSR data produced generally similar conclusions among genotypes within the clusters, confirming the utility of molecular analysis. The studied genotypes were of diverse genetic nature as revealed by their similarity coefficients and may be a good source of variability for sugarcane breeding programmes. Such a wide range of variability among the *S. spontaneum* clones has been well-documented (Al-Janabi *et al.* 1994, Selvi *et al.* 2003). Among the commercial sugarcane hybrids of sugarcane, Harvey and Botha (1996) found 61 to 95% similarity and most of the commercial varieties showed more than 80% similarity in their DNA. Their RAPD data indicated that there had been a gradual decline in DNA diversity (84% reduction) from the early inter-specific crosses to the commercial hybrids, probably as a result of backcrossing and in-breeding strategies used in the previous five to six generations of sugarcane breeding. Nair *et al*. (1999) could observe only 29.31% of genetic distance among 28 tropical and sub-tropical sugarcane genotypes through RAPD and found similarity in large part of genome with lack of parental diversity. The present genotypes of *S. spontaneum* showing similarity coefficients as low as 0.04 and 0.06 through SSR and RAPD may be used in prebreeding programmes in two different crosses in interspecific hybridization programmes for sugarcane improvement.

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