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Genetic studies of "noble cane" for identification and exploitation of genetic markers

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ABSTRACT. Forty genotypes (clones) of sugarcane, including elite lines, commercial cultivars of *Saccharum officinarum* and clones of *S. barberi* were fingerprinted with 50 SSR markers using a PCR-based marker assay. Nei's genetic distances for SSR data were determined and relationships between accessions were portrayed graphically in the form of a dendrogram. Genetic distance values ranging from 0.60 to 1.11 were observed among the 40 sugarcane accessions. The shortest genetic distance of 0.60 was seen between genotypes US-804 and US-130. These two genotypes differed from each other only in 10 bands, with 20 primers. The most dissimilar of the accessions were CP-77-400 and US-133, with a genetic distance of 1.11. SSR fingerprints can help sugarcane breeders to clarify the genetic pedigree of commercial sugarcane varieties and evaluate the efficiency of breeding methods.

Key words: DNA marker; SSR; Genetic distance; Fingerprinting; *Saccharum*

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INTRODUCTION

Saccharum is a complex genus composed of at least six distinct species, *S. officinarum*, *S. barberi*, *S. sinensi*, *S. spontaneum*, *S. robustum*, and *S. edule* (Daniels and Roach, 1987; Naidu and Sreenivasan, 1987; GRIN, 2004) and characterized by high ploidy levels. Being an allopolyploid, modern cultivated sugarcane has approximately 80-140 chromosomes with 8-18 copies of a basic set (i.e., x = 8 or x = 10 haploid chromosome number) (D'Hont et al., 1995; Ha et al., 1999; Ming et al., 2001). *S. officinarum* confers the genes for high sucrose content, low fiber, thick stalks, sparse pubescence, rare flowering, and limited tillering (Ming et al., 2001) and is thought to comprise a large part of the cultivated sugarcane genome. The wild relative, *S. spontaneum*, is credited with imparting the needed pest and disease resistance and abiotic stress tolerance due to its wide eco-geographical adaptive distribution (Sreenivasan et al., 1987) and comprises about 10% of the cultivated sugarcane as evidenced from *in situ* hybridization (D'Hont et al., 1996).

Characterization of germplasm genetic diversity was always seen by plant breeders as the most precise tool in improving the genetic make-up of a cultivated crop species. The first wave of molecular marker applications in plant improvement saw voluminous work concentrated on dissecting and characterizing genetic diversity using random molecular markers. Among the range of DNA-based molecular marker techniques, a promising polymerase chain reaction (PCR)-based technique used extensively for genetic mapping (McIntyre et al., 2001), as well as fingerprinting of sugarcane clones (Piperidis et al., 2000; Pan et al., 2002), is microsatellites or simple sequence repeats (SSRs). Microsatellites have become the marker system of choice due to their high reproducibility, abundance in genome, hypervariability, and co-dominance. Due to their hypervariability and efficiency in polymorphism detection, SSR markers have become ideal for genetic map construction (Devey et al., 1996; Paglia et al., 1998), identification of clones (Dayanandan et al., 1998), identification of species and hybrids, determination of paternity (van de Ven and McNicol, 1996), and marker-assisted selection (Weising et al., 1997).

The present research reports the results of a study on the genetic diversity among 40 genotypes including currently cultivated sugarcane (*S. officinarum*) varieties, elite lines and the wild species *S. barberi* as revealed by the SSR molecular marker system. The objective of the present study was to determine whether polymorphism is sufficient to distinguish sugarcane accessions and to assess the patterns of genetic diversity among a selected group of *Saccharum officinarum* L. in order to provide more information to facilitate breeding programs and to surpass the productivity levels presented today.

MATERIAL AND METHODS

Plant genotypes and DNA extraction

For genetic diversity studies 40 sugarcane genotypes (clones) including elite lines, commercial cultivars of *S. officinarum* and clones of *S. barberi* were selected (Table 1). Parameters like yield potential, maturity trend, ratoonability, salt tolerance, and disease resistance were used as selection criteria for the above mentioned genotypes (data not shown). DNA was extracted from shoot apical meristems, which were ground into fine powder in the presence of liquid nitrogen. Genomic DNA was extracted by the CTAB method (Hoisington et al., 1994) with minor modification for sugarcane.

Table 1. Nei's (1978) measures of genetic identity and genetic distance obtained for 40 sugarcane accessions using 50 simple sequence repeat markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	****																			
2	1.1125	****																		
3	0.8123	1.0781	****																	
4	0.8178	1.1197	0.8103	****																
5	0.9957	1.0781	0.9238	1.0799	****															
6	1.1174	0.9687	1.0938	1.1100	0.8912	****														
7	1.1150	1.1081	1.1119	1.1170	1.1138	0.8649	****													
8	1.1132	1.0174	1.0556	1.0641	1.0556	0.8456	0.9003	****												
9	1.1173	1.0900	0.9747	1.1161	1.1041	1.0172	0.9045	0.9277	****											
10	1.0864	1.0220	0.9957	1.0967	1.0832	0.9913	0.8709	0.9403	0.7809	****										
11	0.9936	0.9431	0.8350	1.0900	1.1132	1.0227	0.7920	0.8713	0.8349	0.6888	****									
12	1.1176	0.9106	1.0196	1.1179	1.1041	1.0579	0.9476	1.0109	0.8679	0.7809	0.7861	****								
13	1.1150	1.0372	1.0229	1.1170	1.1138	0.9629	0.8222	0.9906	0.7608	0.7724	0.7920	0.7064	****							
14	1.1156	1.0738	1.1180	1.1131	1.1113	1.0460	1.0536	1.1126	1.1186	1.0918	1.0316	1.0486	0.8881	****						
15	1.1191	1.1079	1.1144	1.1131	1.1140	0.8345	1.0654	1.0496	1.1193	1.1000	1.0424	1.1193	1.1030	1.0032	****					
16	1.1132	1.0903	1.1141	1.1163	1.3717	1.1110	0.8516	0.0989	1.1142	1.0236	0.8713	1.0500	0.9906	1.0370	0.9679	****				
17	1.1115	1.1187	1.1124	1.1168	1.1158	1.1195	1.0517	1.1198	0.9620	0.9712	1.0281	0.8776	0.8816	1.0630	0.9957	0.9522	****			
18	1.1114	1.1083	1.0486	1.1118	1.1179	1.1139	0.9097	1.1004	0.9844	1.0337	0.8353	0.9438	0.9967	1.0093	1.0192	0.6108	0.9631	****		
19	0.9602	1.0738	0.9140	1.1160	0.8623	1.0460	1.1124	1.1126	1.0841	1.1165	1.0695	1.0117	1.0536	1.1125	1.0411	1.1100	1.1141	1.1150	****	
20	1.0695	0.9298	0.9876	1.1120	0.9425	0.9525	0.8940	1.0352	0.9523	0.9149	0.8277	0.7835	0.8495	0.9727	1.0488	1.0352	0.9562	0.9763	0.8070	****
21	1.0189	0.9461	0.9696	1.0314	1.1141	1.1195	0.9271	0.9076	0.9620	0.9298	0.6978	0.9620	0.9704	1.0255	1.1140	0.9522	1.0583	0.9202	1.0991	0.8294
22	0.9959	0.9089	0.9957	1.1156	0.8997	0.7999	1.0403	0.9403	0.9959	0.9233	0.8916	0.9959	1.0006	1.0918	0.9474	1.1171	1.1162	1.1132	0.8985	0.7837
23	1.1020	0.8307	1.1028	1.1178	1.1119	1.0635	1.0032	0.9003	1.0480	0.9397	0.8571	0.9368	0.9635	1.0676	1.1061	1.0974	1.0909	1.1140	1.0676	0.9824
24	1.1130	1.0741	1.1140	1.1183	1.0032	1.0779	0.9967	1.1004	1.0973	1.0714	0.9284	0.9438	0.9967	1.0846	1.1157	1.1141	1.1137	1.0674	1.0093	0.7567
25	1.1111	1.0198	0.9896	1.1173	1.0360	1.0651	1.1014	1.0476	1.1129	1.1195	0.9549	1.0451	1.1150	1.1158	1.0805	1.1182	1.1132	1.1181	1.0316	0.8729
26	1.0758	0.8872	1.0285	1.1161	1.1144	1.1021	1.0654	1.0881	1.0179	0.9474	0.8738	0.8069	0.8993	1.1174	1.1102	1.1153	1.0724	1.1109	1.0411	0.8520
27	1.1134	0.9822	1.1143	1.3142	1.0805	1.0227	0.9372	1.0064	0.9665	0.8916	0.9104	0.8810	0.9372	1.1060	1.1173	1.0872	1.1044	1.0911	1.1114	0.9568
28	1.1089	0.8644	1.0606	1.1137	1.1033	0.9630	0.9271	0.9949	1.0396	0.9298	0.8061	1.0396	1.0517	0.9867	0.9957	1.0358	1.1161	1.0435	1.0630	0.8294
29	1.1129	0.9248	1.1140	1.1133	1.1140	1.0364	0.9967	1.1179	0.9844	0.9945	0.8831	1.0234	0.9967	1.0846	1.1109	1.1004	1.1181	1.0286	1.0476	0.8054
30	1.1089	1.0213	1.0606	1.1103	1.0161	0.9167	1.0901	1.0358	1.0763	1.0491	0.9877	0.9620	1.0119	1.0630	0.9550	1.1199	1.0954	1.1171	0.8597	0.7826
31	1.1191	0.8872	1.0285	1.1102	1.0285	0.8847	0.9436	1.0496	0.8973	0.8145	0.8263	0.9392	0.9436	1.0411	0.9718	1.0881	1.0348	1.0577	0.9637	0.7081
32	1.1111	0.9822	1.1140	1.1140	1.1124	1.1153	1.1014	1.1154	1.0451	1.0176	0.9549	0.9665	1.0628	1.1060	1.0805	1.1154	1.0281	1.0911	1.1060	0.9158
33	1.1101	1.0313	1.3122	1.3057	1.1109	0.8915	1.1198	1.0542	1.0363	1.0106	1.0401	0.9524	0.9358	0.8922	0.9970	1.0542	1.0667	1.0537	1.1164	0.9325
34	1.0019	1.1118	0.9946	1.0935	1.0422	1.1182	1.0583	1.1193	1.1088	1.0851	0.9893	1.1088	1.1170	1.0591	1.1107	1.0438	1.1123	0.8689	0.9357	0.8461
35	1.0060	0.8514	0.9183	1.0726	0.9183	0.7810	0.8632	0.9290	0.8365	0.7442	0.6884	0.7910	0.8166	0.8598	0.8621	1.0106	0.9669	0.9481	0.9021	0.6558
36	1.1111	1.0912	1.1132	1.1173	1.1197	1.0651	0.9808	1.0476	0.9247	0.9355	0.8143	0.9247	0.8432	0.9513	0.9618	0.9635	0.8061	0.9717	1.0695	0.8729
37	1.1115	0.9844	1.1144	1.1168	1.0161	0.8162	0.8816	0.9949	0.8776	0.7413	0.9014	0.9620	0.8816	1.0630	0.9957	0.9949	0.9797	1.0815	1.0630	0.8737
38	1.3718	1.0741	1.1179	1.3881	1.1140	0.8502	0.9097	1.1004	1.0234	0.9945	1.0130	0.9014	0.9097	0.9279	1.0192	1.1179	0.9631	1.1111	1.0846	0.8514
39	1.1135	0.9999	1.0683	1.1170	0.9255	0.7544	1.0481	0.9466	0.9476	0.8709	0.9808	0.9045	1.0070	1.1173	0.9436	1.1122	1.0517	1.1146	0.9323	0.9363
40	1.1135	1.0372	1.0683	1.1187	1.1133	1.0517	1.0877	0.8516	1.1033	1.0785	0.9372	0.9476	1.0481	1.1165	1.1194	1.1102	1.1172	1.1112	1.0911	0.9769

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Table 1. Continued.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1																				
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18																				
19																				
20																				
21	****																			
22	0.8864	****																		
23	0.9006	1.0521	****																	
24	0.9631	1.0337	1.0751	****																
25	1.0281	0.9355	1.0586	0.7846	****															
26	0.9957	0.9878	0.9606	0.8477	0.9189	****														
27	0.9877	0.8916	0.9429	0.9284	0.9973	0.7759	****													
28	0.8000	0.8408	0.9812	0.8279	0.9014	0.9125	0.9014	****												
29	0.9202	0.8194	1.1101	0.8558	0.9717	0.9374	0.9284	0.6658	****											
30	1.0198	0.6862	1.0909	0.9631	0.9455	0.9550	0.9877	0.8485	0.8753	****										
31	0.9125	0.7650	0.9606	0.8477	0.8738	0.8416	0.8263	1.5972	0.6918	0.7711	****									
32	0.9455	1.0176	0.8571	1.0911	1.1151	0.9618	0.9549	0.9877	0.9717	0.9455	0.8738	****								
33	1.0256	1.0106	1.0040	0.9679	1.1111	1.1180	0.9523	0.9828	1.0117	0.9828	0.9075	0.8554	****							
34	0.9770	1.0463	1.1167	0.9615	1.0723	1.1133	1.1158	0.9770	1.0459	1.1160	0.9918	1.0723	0.9953	****						
35	0.8404	0.7442	0.9114	0.8632	0.8856	0.8621	0.8856	0.6911	0.7690	0.8404	0.6042	0.8406	0.8100	0.8619	****					
36	0.9455	1.0562	0.9429	0.9717	1.1151	0.8738	0.9973	0.9455	1.0130	0.9014	0.8263	0.8637	0.9051	1.0723	0.8406	****				
37	1.0583	0.9298	1.0191	0.9631	1.1044	1.0724	0.9455	0.8944	0.9631	1.0198	0.7711	1.1044	0.8910	1.0595	0.7938	0.9014	****			
38	1.1181	1.0337	1.0751	0.8558	1.0528	0.9792	0.9284	0.8753	0.9021	0.9202	0.7991	1.0130	0.8226	1.1169	0.7690	0.8831	0.7776	****		
39	1.1172	0.8231	1.0413	1.0375	0.9808	1.0654	1.0226	0.9704	0.9542	0.8816	0.8528	1.1014	1.0681	1.0984	0.7671	1.1014	0.8816	0.8629	****	
40	0.9704	1.0006	1.0032	0.9967	0.9808	0.9436	1.0628	0.9271	1.0375	0.8816	0.9859	1.0628	1.1157	1.1156	0.9901	0.9808	1.1172	0.9967	0.9193	****

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PCR amplification

PCR conditions were optimized in a Gene Amp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR for random amplified polymorphic DNA (RAPD) analysis was performed in a 20-µL reaction volume containing 3 µL d3H_2O, 2.0µL 10X PCR buffer, 2 µL $MgCl_2$, 6.8 µL of each dNTP (Fermentas Inc., Hanover, MD, USA), 0.2 µL Taq DNA polymerase (Fermentas), 3.0 µL template DNA and 3 µL of both forward and reverse primers in each reaction mixture. The reactions were subjected to the following profile. Initial denaturation at 94°C for 5 min, followed by 35 cycles, each of which consisted of 1-min denaturation at 94°C, 1-min annealing at 56°C and 2-min extension at 72°C, with a final extension at 72°C for 10 min. Primers that did not show any amplification at 56°C were submitted to annealing temperatures from 50-55°C. Amplification products were mixed with 3 µL 6X loading dye (0.25% bromophenol blue, 0.25% xylene cyanole, and 40% sucrose) spun for a few minutes in a centrifuge before loading. PCR products (7 µL) were loaded in each well of 3.0% agarose gel made with 1.0X TBE buffer and 0.5 µg/mL ethidium bromide, and electrophoresed at 90 W for 2 h. The gels were dried and photographed under UV.

All segregating bands that were well resolved and unambiguous were scored for the presence (1) or absence (0) in the 40 genotypes.

Data analysis

The data on bands generated by the 50 primers were selected for analysis of genetic diversity (Table 2). The bands were counted by starting from the top and ending with the bottom of the lanes. All segregating bands that were well resolved and unambiguous were scored for the presence (1) or absence (0) in the 40 genotypes. The data of the primers were used to estimate the dissimilarity on the basis of number of unshared amplified products and a dissimilarity matrix was generated using Nei’s similarity indices (Nei, 1972). In addition, population relationships were inferred using the unweighted pair group of arithmetic means (UPGMA) clustering method using the Popgen software (version 3.5).

RESULTS

SSR polymorphism

The polymorphism rate was estimated to be 94.25%: 164 of 174 fragments were polymorphic with 50 primers used among the 40 sugarcane accessions. The remaining 10 bands from the 40 accessions were monomorphic. In the present study, the 40 sugarcane accessions appeared to show difference/variability with the 50 primers used. Although none of the primers were individually informative enough to differentiate all the accessions, highly polymorphic profiles were obtained from the sugarcane microsatellite primers No. 035 (SMs035) and SMs040 while five primer pairs (SMs046, SMs047, SMs048, SMs049, and SMs050) were found to be monomorphic. The banding pattern of the primers is shown in Figure 1. Therefore, it may be concluded from the present results that SSRs can be used for identification of genetic diversity and the relationship between the members of the complex.

Table 2. Name of the primers used and DNA polymorphism detected in sugarcane genotypes.

Sr.#	Primer name	Left sequence	Right sequence	Size (bp)	Total No. of fragments generated	No. of polymorphic fragments	Percentage of polymorphic fragments	Annealing temperature (°C)
1	SMs001	GGTTTGTTACTCTACTCCCGT	GGTTTGTTACTCTACTCCCGT	600-2000	5	4	80.0	55
2	SMs002	CATCTGCTCCCTCTTCCT	TGAGCAAAGAAAGAGAAGTAGTC	550-900	3	3	100.0	55
3	SMs003	CTCTGCGGCTTGGTCCTG	CATCCTCCAAGCATCTGT	400-550	2	2	100.0	52
4	SMs004	CATCTGCTCCCTCTTCCT	TGAGCAAAGAAAGAGAAGTAGTC	400-800	5	5	100.0	55
5	SMs005	CTCTGCGGCTTGGTCCTG	CATCCTCCAAGCATCTGT	400-800	4	3	75.0	54
6	SMs006	CATCTGCTCCCTCTTCCT	TGAGCAAAGAAAGAGAAGTAGTC	500-600	3	3	100.0	55
7	SMs007	CTCTGCGGCTTGGTCCTG	CATCCTCCAAGCATCTGT	400-500	4	4	100.0	54
8	SMs008	CATCTGCTCCCTCTTCCT	TGAGCAAAGAAAGAGAAGTAGTC	400-550	4	4	100.0	55
9	SMs009	CTCTGCGGCTTGGTCCTG	CATCCTCCAAGCATCTGT	400-500	3	3	100.0	50
10	SMs010	CATCTGCTCCCTCTTCCT	TGAGCAAAGAAAGAGAAGTAGTC	500-700	3	3	100.0	55
11	SMs011	AAATGTCTTCGCACTAACC	ACACGCATCGCAAGAAGG	400-500	2	2	100.0	55
12	SMs012	AAGGAGATGCTGATGGAGA	AAGAACACTCAACAGAAGCAC	400-600	4	4	100.0	55
13	SMs013	AAATGTCTTCGCACTAACC	ACACGCATCGCAAGAAGG	300-400	4	3	75.0	55
14	SMs014	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	400-800	5	5	100.0	50
15	SMs015	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	400-600	4	4	100.0	52
16	SMs016	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	400-500	3	3	100.0	50
17	SMs017	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	400-450	2	2	100.0	55
18	SMs018	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	400-600	4	3	75.0	55
19	SMs019	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	300-500	3	3	100.0	53
20	SMs020	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	350-500	4	4	100.0	50
21	SMs021	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	400-600	5	5	100.0	50
22	SMs022	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	300-400	3	3	100.0	55
23	SMs023	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	350-600	4	4	100.0	50
24	SMs024	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	400-450	3	3	100.0	53
25	SMs025	GGCTCCTCCTACTCGTTC	GAGCCTTTGGATGTGGTC	600-1000	5	4	80.0	50
26	SMs026	CTACACATCTCCATTCCACAG	TTTAGGGTTCGTTAGGGTAAG	400-550	3	3	100.0	55
27	SMs027	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-500	3	3	100.0	52
28	SMs028	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	400-800	5	5	100.0	52
29	SMs029	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-500	4	3	75.0	50
30	SMs030	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	300-1000	4	4	100.0	55
31	SMs031	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	550-650	3	3	100.0	55
32	SMs032	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	400-600	3	3	100.0	55
33	SMs033	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-800	5	5	100.0	55
34	SMs034	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	400-550	3	3	100.0	55
35	SMs035	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-850	7	7	100.0	53
36	SMs036	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	300-500	4	4	100.0	53
37	SMs037	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-700	4	3	75.0	50
38	SMs038	TGAAGCCTATCTCTTTGGA	TCCCTTGAACTGGCTGTC	380-500	3	3	100.0	50

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Table 2. Continued.

Sr.#	Primer name	Left sequence	Right sequence	Size (bp)	Total No. of fragments generated	No. of polymorphic fragments	Percentage of polymorphic fragments	Annealing temperature (°C)
39	SMs039	TTCTCGCCCTCCCGCTAC	TTCTCTCCTCCTCCTCTTTC	400-700	4	4	100.0	55
40	SMs040	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	300-800	7	7	100.0	50
41	SMs041	CCCAGTGCTTCCTCTCTC	TAGCACTCCATTCAGCAAA	380-500	3	3	100.0	55
42	SMs042	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	400-500	3	3	100.0	55
43	SMs043	CCCAGTGCTTCCTCTCTC	TAGCACTCCATTCAGCAAA	400-500	4	4	100.0	55
44	SMs044	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	400-600	5	5	100.0	52
45	SMs045	CCCAGTGCTTCCTCTCTC	TAGCACTCCATTCAGCAAA	400	1	1	100.0	55
46	SMs046	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	400	1	0	0.0	50
47	SMs047	CCCAGTGCTTCCTCTCTC	TAGCACTCCATTCAGCAAA	400	1	0	0.0	50
48	SMs048	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	400	1	0	0.0	53
49	SMs049	CCCAGTGCTTCCTCTCTC	TAGCACTCCATTCAGCAAA	400	1	0	0.0	55
50	SMs050	GTTTCTCCACCTCCAACTC	ACAGACACAGGCGGGCGA	400	1	0	0.0	55
Total				-	174	162	-	-
%		-	-	-	3.48%	3.24%	94.25%	-

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Genetic distance between the accessions

The genetic distance for SSR data using 40 sugarcane accessions was constructed based on Nei (1972) as shown in Table 1 and relationships between accessions were portrayed graphically in the form of a dendrogram in Figure 1. Genetic distances ranging from 0.60 to 1.11 were observed among the 40 sugarcane accessions. The lowest genetic distance of 0.60 was seen between genotypes US-804 and US-130.

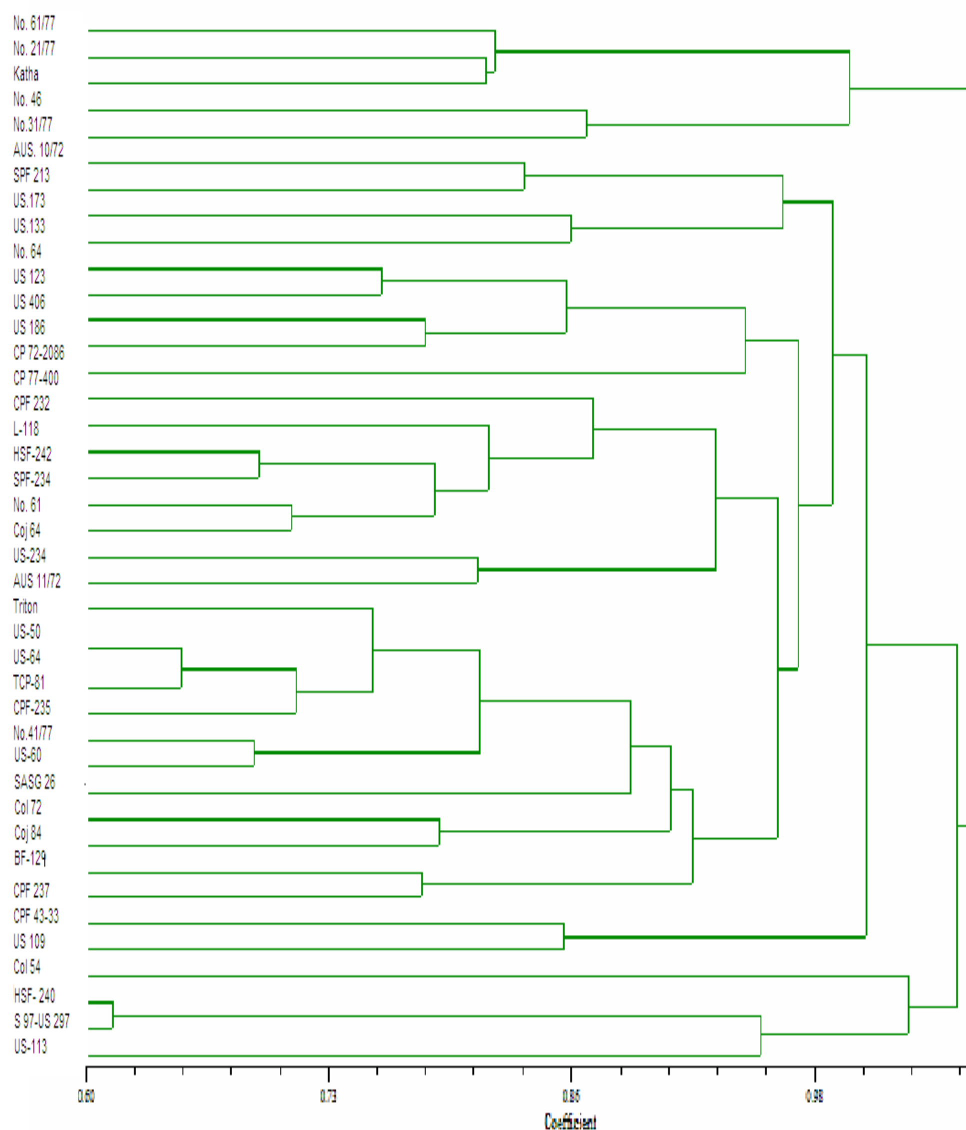

Figure 1. Dendrogram of 40 sugarcane accessions developed from simple sequence repeat data using unweighted pair group of arithmetic means (UPGMA) based on Nei's (1972) genetic distance.

These two genotypes differed from each other only in 10 bands with 20 different primers. The most dissimilar of all the accessions were CP-77-400 and US-133 with a genetic distance of 1.119. The genetic diversity among the sugarcane accessions is shown in Table 3.

Table 3. Different sugarcane accessions used for the study of genetic diversity.

Sr. No.	Genera and species	Cultivar	Origin	Source of collection
1.	*Saccharum barberi*	No. 61/77	Unknown	UAF
2.		AUS-10/72	Australia	UAF
3.		No. 21/77	Unknown	UAF
4.		Katha	India	UAF
5.	*Saccharum officinarum*	No. 46	Unknown	UAF
6.		No. 64	Unknown	UAF
7.		CP-77-400	Canal point	UAF
8.		CP-43-33	Canal point	UAF
9.		CPF-232	Canal point	UAF
10.		L-118	Louisiana	UAF
11.		HSF-242	Unknown	UAF
12.		SPF-234	Brazil	UAF
13.		No. 61	Unknown	UAF
14.		Col-54	Columbia	UAF
15.		CP-72-2086	Canal point	UAF
16.		HSF-240	Unknown	UAF
17.		Coj-64	Early Indian	UAF
18.		S-97-US-297	Unknown	UAF
19.		No. 31/77	Unknown	UAF
20.		AUS-11/72	Australia	UAF
21.		SASG-26	Pakistan	UAF
22.		CPF-235	Canal point	UAF
23.		SPF-213	Unknown	UAF
24.		Col-72	Columbia	UAF
25.		Coj-84	India	UAF
26.		BF-129	Unknown	UAF
27.		CPF-237	Canal point	UAF
28.		Triton	India	UAF
29.		TCP-81	Brazil	UAF
30.		No. 41/77	Unknown	UAF
31.		US-50	USA	AARI
32.		US-173	USA	AARI
33.		US-133	USA	AARI
34.		US-113	USA	AARI
35.		US-64	USA	AARI
36.		US-234	USA	AARI
37.		US-406	USA	AARI
38.		US-186	USA	AARI
39.		US-123	USA	AARI
40.		US-109	USA	AARI

UAF = University of Agriculture, Faisalabad; AARI = Ayub Agricultural Research Institute, Faisalabad.

Clustering pattern

The cluster analysis, based on dissimilarity values, classified all the sugarcane accessions into two major groups (I and II) (Figure 1). The first major group was further grouped into IA and IB. Group IA consisted of three *S. barberi* accessions namely No. 61/77, AUS-10/72 and Katha. Group IB consisted of two accessions, No. 46 and No. 31/77. The second major group was further grouped into IIA and IIB. Group IIA consisted of four accessions, namely Col-54, HSF-240, S-97-US-297, and US-113. Group IIB was again divided into two subgroups, IIB1 and IIB2. Subgroup IIB1 comprised four subgroups IIB1a1, IIB1a2, IIB1a3, and IIB1a4.

Subgroup IIB1a1 consists of four sugarcane accessions, namely AUS-10/72, TCP-81, US-173, and US-133. Subgroup IIB1a2 consists of five sugarcane accessions, namely No. 64, CP-72-2086, US-406, US-186, and US-123. Subgroup IIB1a3 was further divided into two subclusters, IIB1a3-1 and IIB1a3-2. Group IIB1a3-1 consists of two sugarcane accessions, namely Coj-64 and US-234. Group IIB1a3-2 consists of six sugarcane accessions, namely CP-77-400, CPF-232, L-118, HSF-242, SPF-234, and No. 61. Subgroup IIB1a4 was further divided into three subclusters, IIB1a4-1, IIB1a4-2 and IIB1a4-3. Cluster IIB1a4-1 consists of two sugarcane accessions, namely BF-129 and CPF-237. Cluster IIB1a4-2 consists of two sugarcane accessions, namely Col-72 and Coj-84. Cluster IIB1a4-3 consists of 8 sugarcane accessions, namely AUS-11/72, Triton, US-173, US-64, TCP-81, CPF-235, No. 41/77, and SASG-26.

DISCUSSION

As genetic markers, SSRs are usually considered co-dominant markers although several issues regarding their use have been recorded, including the tendency for Taq polymerase to add an adenosine nucleotide to the 3’ end of products; the inability of the marker to distinguish between homology of fragments that run at the same band size, and mutations in the binding region of microsatellite primers resulting in the loss of the PCR product (null-alleles) (Hu, 1993; Callen et al., 1993). These issues are compounded in the highly polyploid sugarcane genome, particularly where the difficulty in distinguishing alleles from homoeologous chromosomes makes it difficult to determine heterozygosity or homozygosity at any particular locus. Hence, for the purposes of this study, SSRs have been considered dominant markers. It is however, the intention of this discussion to investigate the utility of SSRs for the identification of genetic diversity and the relationships between members of the complex. Hence, an approach that looks at genetic distances between individuals, rather than any attempt at elucidating the evolutionary history, was adopted.

The genetic distance of 40 accessions ranging from 0.60 to 1.11 with an average of 0.85 suggested that the level of genetic diversity among the sugarcane accessions is high. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed genetic diversity as well (Selvi et al., 2003; Cordeiro et al., 2003). Selvi et al. (2003) revealed a broad range (0.324-0.8335) of pair-wise similarity values when tested on 30 or 40 commercial sugarcane cultivars.

The complex banding patterns encountered in sugarcane is due to its high level of polyploidy and heterozygosity as compared to other genera. Reports from the International Sugarcane Microsatellite Consortium show the amplification of several fragments per clone with a theoretical maximum of 12 fragments.

Furthermore, isozyme analysis has revealed complex banding patterns in relation to their high ploidy level (Glaszmann et al., 1989). In the present study, bands were produced in the range of 2-7, which is much lower than the above mentioned studies. In spite of the high polyploidy and heterozygosity of the *Saccharum* genome, few primers amplified a single discrete band across the members of the *Saccharum* complex, suggesting that these allelic regions or primer binding sites are highly conserved and no SSR expansion or contraction has taken place during the evolution of *Saccharum officinarum* and *Saccharum barberi*. Another reason for fewer bands being produced is that the primers range in size from 300-420 bp.

A high degree of similarity between *S. officinaum* and *S. barberi* as revealed in the present study has been documented by other marker systems (Glaszmann et al., 1989; Nair et

al., 1999). The proximity between the two species is expected, since *S. barberi* is considered to be the progenitor species of *S. officinarum*.

The observation made in this study is supported by the results of RFLP and RAPD profiles where *S. barberi* and *S. sinense* share the nuclear DNA pattern of *S. officinarum* and *S. spontaneum* (Lu et al., 1994; Nair et al., 1999). *S. barberi* and *S. sinense* are thought to be of secondary origin derived through hybridization between *S. officinarum* and *S. spontaneum* (Daniels and Roach, 1987).

In the present study, *S. barberi* clones showed low genetic distance from *S. officinarum* clones as four *barberi* clones do not form distinct clusters but cluster with other *officinarum* clones.

AUS-10/72, which belongs to *barberi* and is Australian in origin, along with three accessions, TCP-81, US-173 and US-133, of Brazilian and USA origins, constituted the cluster IIB1a1. The other three *barberi* clones, namely No. 61/77, AUS-10/72 and Katha, fall in the single cluster IA.

Although a high range of dissimilarity (0.60-1.119) was estimated among the genotypes, the genetic distances between the *barberi* and *officinarum* clones are not high.

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