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Phylogenetic relationships among *Saccharum* clones in Pakistan revealed by RAPD markers

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ABSTRACT. Forty sugarcane genotypes (clones), including elite lines, commercial cultivars of *Saccharum officinarum* and *S. barberi* clones, were fingerprinted with 30 RAPD markers, using a PCR-based marker assay. The genetic distance for RAPD data was determined according to Nei, and relationships between accessions were graphed in a dendrogram. Genetic distance values ranging from 16.2 to 86.3% were observed among the 40 sugarcane accessions. The lowest genetic distance was found between genotypes US-406 and US-186. These two genotypes differed from each other in only 25 bands with 15 different primers. Genotypes Col-54 and CP-72-2086 were the second most similar group, with a genetic distance of 19.46%. The most dissimilar of all the accessions were CP-77-400 and US-133, with a genetic distance of 86.3%. RAPD fingerprints help sugarcane breeders clarify the genetic pedigree of commercial sugarcane varieties and can be used to evaluate the efficiency of conventional breeding methods.

Key words: DNA marker; RAPD; Genetic distance; Sugarcane; Fingerprinting

INTRODUCTION

Sugarcane is a large perennial grass belonging to the genus *Saccharum*, which is an important component of the grass family Poaceae and the tribe Andropogoneae. Six *Saccharum* species, namely *Saccharum officinarum*, *S. robustum*, *S. spontaneum*, *S. barberi*, *S. sinense*, and *S. edule*, along with *Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya* are so closely related that Mukherjee (1957) referred to them as the "*Saccharum* complex".

The evolution of cultivated sugarcane could have resulted through several stages of intergeneric and interspecific hybridization involving the above core taxa, followed by polyploidy and natural- and human-mediated selection. In sugarcane, the assessment of genetic diversity existing among the species and related genera is particularly important, since a major breakthrough in sugarcane varietal improvement was obtained through distant hybridization. Although varietal diversification is apparent among the current commercial varieties, an assessment of their genetic diversity is lacking. The extent of genetic diversity among the genetic materials has been estimated by adopting various methods over a period of time using a wider range of simply and complexly inherited traits. Although morphological and isozyme markers have been employed in assessing the underlying genetic diversity of a species, the accuracy of such assessment is questionable. Considering the problems associated with morphological and isozyme markers, research on genetic diversity analysis has searched for alternative tools. Advances in molecular biology techniques have provided the basis for uncovering virtually unlimited numbers of DNA markers. During the last decade, several molecular marker systems such as restriction fragment length polymorphism (RFLP), microsatellite, random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) were developed and used for the estimation of genetic diversity (Wang et al., 1992; Stiles et al., 1993; Orozco-Castillo et al., 1994; Nakajima et al., 1998; Hokanson et al., 1998; Aggarwal et al., 1999; Angiolillo et al., 1999; Barker et al., 1999).

Polymerase chain reaction (PCR)-based methods such as RAPD are increasingly being used in the analysis of genetic diversity in crop plants, because of the relative ease with which PCR assays can be carried out compared to RFLPs. Both genomic and organellar RFLP along with PCR-based markers have been used to study the variability and diversity among *Saccharum* species and hybrid populations (D'Hont et al., 1993; Al-Janabi et al., 1994; Lu et al., 1994; Sobral et al., 1994; Burnquist et al., 1995; Harvey and Botha, 1996; Nair et al., 1999). These studies have been useful in assessing the genetic diversity among *Saccharum* species and hybrids.

This paper reports the results of a study of the genetic diversity among 40 genotypes including currently cultivated sugarcane varieties, elite lines and the wild *S. barberi* species, as revealed by RAPD molecular markers. RAPD fingerprints help sugarcane breeders in the identification of mislabeled varieties during field trials (Lande and Thompson, 1990), marker-assisted selection of true cross progeny at seedling stage, and evaluation of the extent of self-pollination in conventional crossing procedure (Stuber et al., 1999).

MATERIAL AND METHODS

Plant genotypes and DNA extraction

Forty sugarcane genotypes (clones), including elite lines, commercial cultivars of *S. officinarum* and clones of *S. barberi* were selected for the study of genetic diversity (Table 1).

The parameters 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 liquid nitrogen. Genomic DNA was extracted by the CTAB method (Hoisington et al., 1994) with minor modifications for sugarcane.

Table 1. 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		AUS10/72	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	Colombia	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		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	Colombia	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 Agriculture Research Institute.

PCR amplification

PCR conditions were optimized in a Gene Amp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR for RAPD analysis was performed in a 25-µL reaction volume containing 8.3 µL d_3H_2O, 2.5 µL 10X PCR buffer, 2.5 µL gelatin, 3 µL $MgCl_2$, 4 µL of each dNTP (Fermentas Inc., MD, USA), 0.2 µL *Taq* DNA polymerase (Fermentas),

2.5 µL template DNA and 2 µL of each primer. The reaction mixes were subjected to the following protocol: initial denaturation at 94°C for 5 min, followed by 35 cycles, each consisting of 1 min denaturation at 94°C, 2 min annealing at 36°C and 1 min extension at 72°C, with a final extension at 72°C for 5 min. Amplification products were mixed with 3 µL 6X loading dye (0.25% bromophenol blue, 0.25% xylene cyanole, and 40% sucrose) spun for few minutes in a centrifuge before loading. The PCR products (7 µL) were loaded in each well of a 1.2% agarose gel made with 0.5X 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 bands that were well resolved and unambiguous were scored for presence (1) or absence (0) in the 40 genotypes.

Data analysis

The data on bands generated by the 30 primers were selected for the analysis of genetic diversity. The bands were counted by starting from the top of the lanes to their bottom. The data of the primers were used to estimate dissimilarity based on the number of unshared amplified products, and a dissimilarity matrix was generated using Nei's similarity indices (Nei, 1978). In addition, population relationships were inferred using the unweighted pair group method with arithmetic mean (UPGMA) clustering method using the Popgen software (version 3.5).

RESULTS

RAPD polymorphism

Ninety-two and five percent polymorphic bands were estimated since 219 of 238 fragments were polymorphic with 30 primers used among the 40 sugarcane accessions. The rest of the 19 bands were monomorphic in the 40 accessions. In the present study, the 40 sugarcane accessions appeared to show a difference/variability with the 30 primers used. Although none of the primers was individually so informative as to differentiate all accessions, highly polymorphic profiles were obtained with primers such as GL Decamer A-5, GL Decamer A-9, GL Decamer B-3, GL Decamer B-11, GL Decamer B-I7, and GL Decamer D-1. Therefore, it may be concluded from the present results that RAPD markers can be used for the identification of sugarcane accessions.

Genetics distance between the accessions

The genetic distance for RAPD data using 40 sugarcane accessions was constructed according to Nei (1978), and relationships between accessions were portrayed graphically in the form of a dendrogram in Figure 1. Genetic distance values ranging from 16.21 to 86.33% were observed among the 40 sugarcane accessions. The lowest genetic distance was 16.21 as seen in genotypes US-406 and US-186. These two genotypes differed from each other only in 25 bands with 15 different primers. The genotypes Col-54 and CP-72-2086 were the second similar group with a genetic distance of 19.46%. The most dissimilar of all the accessions was CP-77-400 and US-133 with a genetic distance of 86.33%.

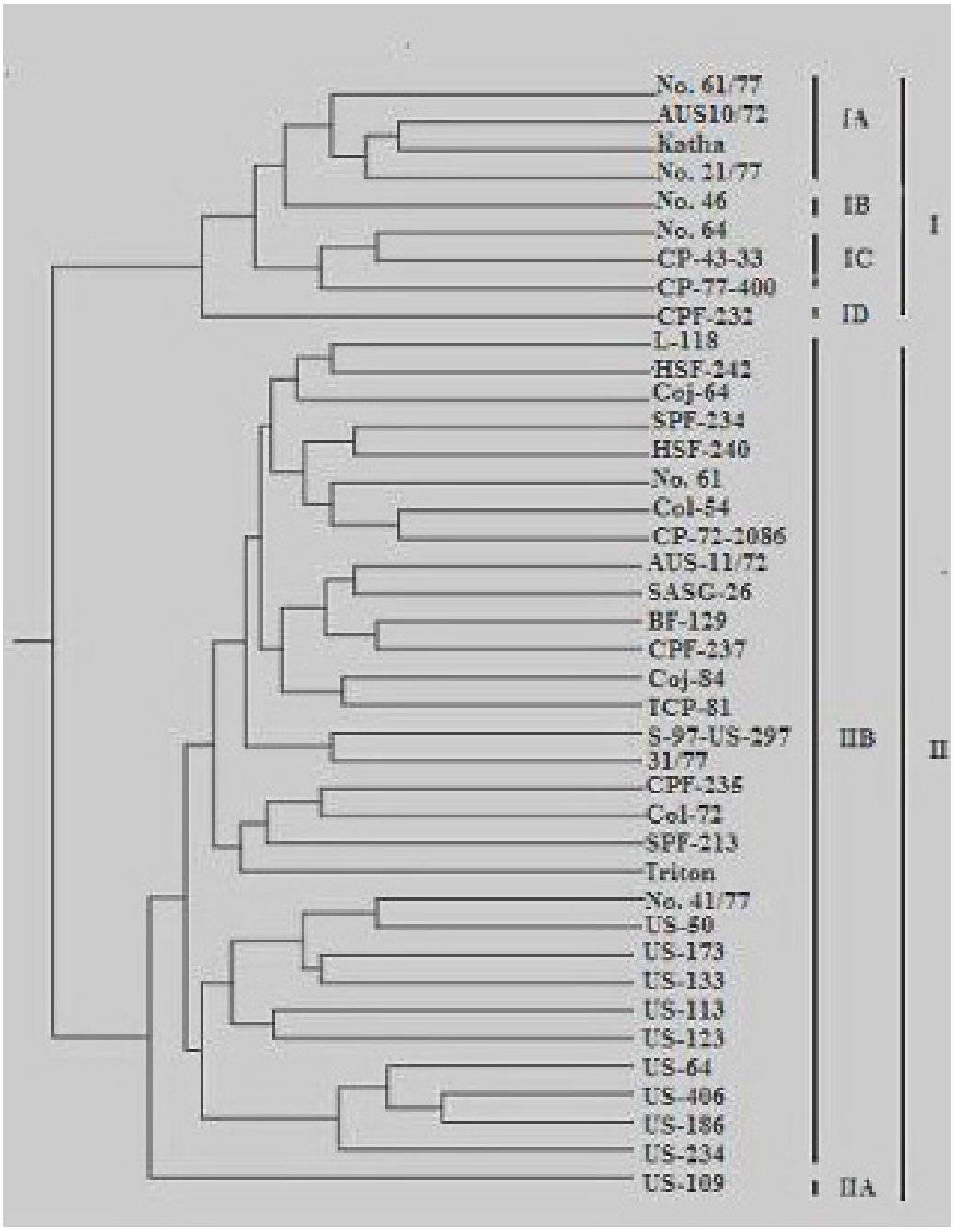

Figure 1. Dendrogram of 40 sugarcane accessions constructed from RAPD data using unweighted pair group method with arithmetic mean (UPGMA) based on Nei's (1978) genetic distance.

Clustering pattern

The cluster analysis based on dissimilarity values classified all sugarcane accessions into two major groups (I and II) (Figure 1). The first major group was further divided into four clusters IA, IB, IC, and ID. Cluster IA consisted of four *S. barberi* accessions, namely No. 61/77, AUS10/72, No. 21/77, and Katha, while Cluster IB consisted of only one *S. officinarum* accession, namely No. 46. Cluster IC consisted of three accessions, namely No. 64, CP-77-400 and CP-43-33. Cluster ID consisted of only one accession, namely CPF-232. The second major group was further divided into Groups IIA and IIB. Group IIA consisted of only one genotype, namely US-109. Group IIB was again divided into seven sub-groups, IIB_1, IIB_2, IIB_3, IIB_4, IIB_5, IIB_6, and IIB_7. Sub-group IIB_1 consisted of four elite lines US-64, US-234, US-406, and US-186, while sub-group IIB_2 was further divided into two sub-clusters. Group IIB_2a comprised two elite lines, US-113 and US-123. Group IIB_2b comprised four elite lines No. 41/77, US-50, US-173, and US-133. Sub-group IIB_3 consisted of four sugarcane accessions, namely CPF-235, SPF-213, Col-72, and Triton. Sub-group IIB_4 consisted of two sugarcane accessions, namely S-97-US-297 and No. 31/77. Sub-group IIB_5 was further divided into two sub-clusters. Group IIB_5a comprised two accessions, namely Coj-84 and TCP-81. Group IIB_5b comprised four accessions, namely AUS-11/72, SASG-26, BF-129, and CPF-237. Sub-group IIB_6 was further divided into two sub-clusters. Group IIB_6a comprised three accessions, namely No. 61, Col-54 and CP-72-2086. Group IIB_6b comprised two accessions, namely SPF-234 and HSF-240. Sub-group IIB_7 consisted of three sugarcane accessions, namely L-118, HSF-242 and Coj-64.

DISCUSSION

For achieving improved productivity in the sugarcane crop, it is essential to maintain a high degree of genetic diversity among the commercial varieties and breeding populations. Although diversification is apparent among the current germplasm collection, an assessment of its genetic diversity is lacking. The present investigation reports the results of a study on the genetic diversity among 40 accessions of sugarcane belonging to *S. officinarum* L. and *S. barberi* as revealed by RAPD. By using RAPD as genetic markers, as high as 92.05% polymorphic bands were detected in 40 accessions of sugarcane. Nair et al. (2002) investigated genetic diversity in prominent Indian sugarcane varieties with 63.74% polymorphism. Burner et al. (1997) compared the genetic diversity of North American and an Old World member of *Saccharum* with 31.44% polymorphism across the taxa. Polymorphism revealed by RAPD could be a result of nucleotide changes at the primer annealing site or due to addition or deletion between two priming sites, which results in different lengths of the amplification products (Williams et al., 1990).

Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990). The genetic distance of 40 accessions ranging from 0.16 to 0.86 (Table 2) with an average of 0.51 suggests that the level of genetic diversity among the sugarcane accessions is moderate. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm also showed genetic diversity (Arceneaux, 1967; Harvey et al., 1994; Harvey and Botha, 1996). Harvey and Botha (1996) reported similarities as high as 77-95% among 20 elite varieties.

Table 2. Nei's (1978) measures of genetic identity and genetic distance obtained for 40 sugarcane accessions using 30 RAPD markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	****																			
2	0.2197	****																		
3	0.2630	0.2197	****																	
4	0.2542	0.1946	0.2179	****																
5	0.2900	0.2809	0.3270	0.2283	****															
6	0.2630	0.2900	0.3176	0.3083	0.2719	****														
7	0.3083	0.2991	0.3083	0.3176	0.3176	0.2368	****													
8	0.2991	0.3460	0.3365	0.2900	0.2900	0.2113	0.2719	****												
9	0.3655	0.3753	0.4261	0.2991	0.3176	0.3270	0.3557	0.2900	****											
10	0.7000	0.7138	0.7000	0.6597	0.6864	0.5960	0.6864	0.6210	0.4686	****										
11	0.6210	0.7138	0.6210	0.5837	0.6597	0.5245	0.6337	0.5716	0.4686	0.2455	****									
12	0.6864	0.7000	0.6337	0.6466	0.5960	0.6337	0.6466	0.6864	0.5018	0.2542	0.2900	****								
13	0.7563	0.6864	0.7278	0.7138	0.6864	0.7563	0.8008	0.7857	0.6084	0.2991	0.3365	0.2542	****							
14	0.7000	0.6084	0.6729	0.5837	0.6337	0.6210	0.6084	0.7278	0.4906	0.2455	0.2630	0.2719	0.2455	****						
15	0.7000	0.6864	0.7000	0.6337	0.6084	0.7278	0.6337	0.7278	0.5131	0.3176	0.2991	0.2719	0.2455	0.1946	****					
16	0.6337	0.6729	0.6337	0.6466	0.6466	0.6597	0.6210	0.7138	0.5245	0.2900	0.3083	0.2283	0.3270	0.2719	0.2029	****				
17	0.6864	0.8160	0.7138	0.6466	0.6466	0.6597	0.7278	0.6337	0.5478	0.2542	0.2900	0.2809	0.3853	0.2719	0.2719	0.2630	****			
18	0.5960	0.6597	0.7000	0.5837	0.6597	0.6210	0.6597	0.6466	0.6084	0.3365	0.3176	0.3270	0.3557	0.2630	0.2991	0.3460	0.3083	****		
19	0.6210	0.6597	0.6729	0.5596	0.6597	0.6729	0.6864	0.6466	0.5361	0.3557	0.2809	0.2542	0.2809	0.2630	0.2809	0.3083	0.2900	0.2455	****	
20	0.5960	0.7138	0.7000	0.6084	0.6597	0.6210	0.6337	0.5716	0.5837	0.3365	0.2630	0.2900	0.3176	0.2991	0.2455	0.2719	0.2542	0.2991	0.2455	****
21	0.6466	0.6597	0.6210	0.5596	0.6337	0.6466	0.7138	0.6210	0.5361	0.2809	0.2455	0.3270	0.3953	0.2991	0.2809	0.3270	0.2542	0.3176	0.2809	0.2283
22	0.6084	0.5716	0.6337	0.5478	0.6210	0.5837	0.6729	0.6597	0.6466	0.3853	0.2719	0.3753	0.4055	0.3460	0.3853	0.3176	0.3557	0.3460	0.3655	0.3083
23	0.6597	0.6466	0.6084	0.5478	0.5960	0.6597	0.6466	0.6864	0.5716	0.4261	0.3655	0.3557	0.3655	0.3460	0.3270	0.3557	0.3365	0.4055	0.4261	0.3460
24	0.7278	0.7138	0.7000	0.6084	0.6597	0.7000	0.7419	0.5960	0.6048	0.2809	0.2630	0.3270	0.3953	0.3176	0.2809	0.2900	0.2900	0.4157	0.3557	0.2809
25	0.6729	0.6084	0.6210	0.5837	0.5837	0.6210	0.7419	0.6466	0.4906	0.3557	0.3557	0.3083	0.3176	0.2991	0.2630	0.3270	0.3083	0.3365	0.2991	0.2991
26	0.7857	0.7419	0.7278	0.6597	0.6864	0.7563	0.8315	0.7857	0.5596	0.3365	0.3557	0.3083	0.2809	0.3176	0.2809	0.3270	0.3460	0.4157	0.2991	0.2991
27	0.5960	0.6337	0.6466	0.5596	0.5361	0.6729	0.6597	0.6466	0.4906	0.2809	0.2991	0.2542	0.2630	0.2630	0.2809	0.2719	0.2719	0.3365	0.2630	0.2283
28	0.6864	0.6466	0.6597	0.6210	0.5960	0.6597	0.6729	0.5837	0.5245	0.3270	0.3083	0.2991	0.3460	0.3655	0.3655	0.3665	0.3557	0.4055	0.3655	0.3083
29	0.6337	0.6466	0.6337	0.6210	0.6466	0.6337	0.7000	0.6597	0.5716	0.3655	0.3270	0.2991	0.3270	0.2900	0.2900	0.2809	0.2455	0.3853	0.3655	0.3270
30	0.7278	0.7419	0.6729	0.7419	0.7138	0.7278	0.8008	0.7278	0.6864	0.3753	0.3176	0.3270	0.3176	0.2991	0.2991	0.3083	0.3460	0.4157	0.4157	0.3365
31	0.6466	0.8008	0.6729	0.7138	0.6597	0.6466	0.7419	0.6466	0.5361	0.3365	0.2630	0.2900	0.3365	0.2809	0.3557	0.3270	0.2900	0.4157	0.3557	0.3176
32	0.6337	0.6729	0.6597	0.6466	0.6729	0.6864	0.6466	0.7419	0.5960	0.3270	0.3853	0.2809	0.2900	0.2542	0.3460	0.2809	0.3557	0.2900	0.3270	0.3083
33	0.7000	0.8315	0.7563	0.6864	0.7419	0.7563	0.8633	0.7857	0.6597	0.3557	0.4157	0.3083	0.3557	0.3557	0.3557	0.3083	0.3083	0.3557	0.3557	0.3557
34	0.6864	0.8473	0.7138	0.7857	0.8160	0.7709	0.7278	0.7709	0.6210	0.4055	0.4261	0.3753	0.3853	0.3853	0.4471	0.3753	0.3953	0.3460	0.4261	0.4055
35	0.6337	0.7278	0.6864	0.7563	0.7857	0.5837	0.7563	0.5837	0.5478	0.3038	0.3460	0.3365	0.4055	0.4261	0.4471	0.3753	0.3365	0.4471	0.4261	0.3083
36	0.7419	0.7857	0.8315	0.6210	0.6210	0.6337	0.7857	0.6337	0.5478	0.3270	0.4686	0.2991	0.4471	0.4261	0.3853	0.3753	0.3365	0.3853	0.3853	0.3270
37	0.6864	0.7000	0.6597	0.5960	0.7278	0.5837	0.6729	0.6084	0.5478	0.3038	0.3460	0.2630	0.4261	0.3853	0.3853	0.3176	0.3365	0.3655	0.3655	0.3270
38	0.6337	0.6729	0.6597	0.5960	0.6210	0.6084	0.7000	0.6084	0.5478	0.3853	0.3655	0.2991	0.4261	0.4055	0.3853	0.3753	0.3365	0.3460	0.4261	0.3270
39	0.8160	0.7419	0.7278	0.6864	0.7138	0.7000	0.8315	0.7278	0.6337	0.3365	0.4157	0.3270	0.3557	0.4366	0.3557	0.3853	0.3460	0.4366	0.4157	0.3953
40	0.7709	0.7857	0.7709	0.7000	0.6466	0.6864	0.8473	0.7709	0.5245	0.3853	0.4471	0.3753	0.3460	0.4261	0.4055	0.3753	0.3365	0.4055	0.3853	0.4471

Continued on next page

Table 2. Continued.

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1																				
2																				
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18																				
19																				
20																				
21	****																			
22	0.2719	****																		
23	0.3270	0.2630	****																	
24	0.2630	0.2542	0.3270	****																
25	0.2833	0.3460	0.3270	0.2809	****															
26	0.2283	0.3853	0.3270	0.2991	0.2991	****														
27	0.2455	0.3460	0.3460	0.2809	0.2283	0.2113	****													
28	0.3083	0.2809	0.3176	0.3460	0.3460	0.3270	0.2542	****												
29	0.3083	0.3176	0.2991	0.3270	0.2368	0.3270	0.2542	0.2809	****											
30	0.3176	0.2900	0.3083	0.3365	0.3365	0.3176	0.3365	0.3655	0.2719	****										
31	0.2991	0.4261	0.3083	0.3953	0.3753	0.3176	0.3365	0.3270	0.3270	0.2113	****									
32	0.3460	0.3365	0.3557	0.3853	0.3270	0.3853	0.2542	0.3557	0.2991	0.2900	0.2542	****								
33	0.3176	0.3853	0.3460	0.3753	0.3176	0.2809	0.2809	0.3270	0.3083	0.2630	0.2630	0.2542	****							
34	0.4471	0.3753	0.3953	0.4471	0.4471	0.4686	0.3853	0.3953	0.4366	0.2719	0.3655	0.3176	0.2900	****						
35	0.3270	0.4157	0.3953	0.4261	0.3655	0.3655	0.3655	0.3953	0.3176	0.3270	0.3083	0.3953	0.3460	0.4366	****					
36	0.3853	0.4366	0.4157	0.4055	0.3270	0.3853	0.3655	0.4578	0.4157	0.3853	0.4055	0.4578	0.3270	0.3753	0.2630	****				
37	0.2719	0.3176	0.3557	0.3853	0.3460	0.3460	0.3853	0.3557	0.3753	0.3270	0.3270	0.3753	0.2900	0.3753	0.2113	0.2630	****			
38	0.3038	0.2991	0.2809	0.4055	0.3038	0.4055	0.3853	0.3557	0.3176	0.3270	0.3038	0.3753	0.3083	0.3176	0.1946	0.1946	0.1621	****		
39	0.3365	0.2900	0.3655	0.3365	0.3176	0.3176	0.3557	0.3853	0.3270	0.2455	0.4578	0.3270	0.2991	0.2900	0.3270	0.2900	0.2719	0.2900	****	
40	0.3853	0.4366	0.3953	0.5131	0.3460	0.3270	0.3270	0.4796	0.3753	0.3853	0.4261	0.3753	0.2719	0.3953	0.3753	0.3753	0.3365	0.3365	0.3270	****

In the present study, the four *S. barberi* clones used formed clusters with *S. officinarum* clones, instead of forming distinct/separate clusters, supporting the assumption of Pan et al. (2003) that sugarcane cultivars (*Saccharum* hybrids) are aneupolyploid hybrids of *S. officinarum*, *S. barberi*, *S. sinense*, and S. *robustum*. Nair et al. (1999) hypothesized that only two species, *S. robustum* and *S. spontaneum*, are the progenitors of modern sugarcane, that *S. officinarum* may be derived from *S. robustum*, and that *S. barberi* and *S. sinense* are cultivated forms of interspecific hybrids between *S. spontaneum* and *S. officinarum*. The above hypothesis is supported by the present study as *S. barberi* clones (No. 61/77, AUS10/72, No. 21/77, and Katha) and *S. officinarum* clones formed a different cluster in Group I. However, the genetic distance between *S. barberi* clone No. 61/77 and another clone in that group was 0.21, 0.25, 0.26, 0.29, 0.30, and 0.36, respectively.

Thus, the *S. officinarum* varieties and *S. barberi* clones have genetic distances in the range of 0.21 to 0.36, where the level of genetic diversity between these sugarcane accessions is somewhat moderate. Other accessions are grouped under different clusters and US-109 is the most distinct accession, forming a separate cluster. The mean genetic distance among the 40 accessions in this study was 51%, implying that the genetic diversity among the genotypes is limited. This is probably due to the lack of parental diversity, where a few clones are themselves related, contributing to the parentage of these varieties. Thus, conscious efforts are to be made to diversify the parental genetic base to ensure high genetic variability among the cultivated varieties and elite lines. New sources from the interspecific/intergeneric hybrid gene pool need to be used along with proven parents to generate the variability that will be both commercially viable and genetically diverse. Elite lines can also be further improved by arranging their cross with *S. barberi*, as this wild species contains high tillering ability and disease resistance.

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