Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

DNA profiling of sugarcane genotypes using randomly amplified polymorphic DNA

S. Tabasum[1], F.A. Khan[2], S. Nawaz[2], M.Z. Iqbal[3] and A. Saeed[2]

[1]College of Agriculture, University of Sargodha, Sargodha, Pakistan
[2]Department of Plant Breeding and Genetics,
University of Agriculture, Faisalabad, Pakistan
[3]Ayub Agriculture Research Institute, Faisalabad, Pakistan

Corresponding author: A. Saeed
E-mail:drasifpbg@gmail.com

Genet. Mol. Res. 9 (1): 471-483 (2010)
Received December 3, 2009
Accepted January 4, 2010
Published March 16, 2010

ABSTRACT. DNA profiles of 40 sugarcane genotypes were constructed with 30 RAPD markers. Sugarcane genotypes of both *Saccharum officinarum* and *S. barberi* were included in this study. Multiple alleles were detected from each RAPD; there was a high level of polymorphism. On average, 7.93 alleles were produced per primer, giving a total of 238 alleles. The genetic distances between these genotypes were assessed with the POPGENE DNA sequence analysis software. A dendrogram was constructed from these data; cultivated species of sugarcane formed clusters with *S. barberi* genotypes. The 40 genotypes were clustered into two main groups; genetic distances ranged from 20.29 to 64.66%. These RAPD fingerprints will help sugarcane breeders to evaluate the efficiency of current conventional breeding methods and will help characterize the genetic pedigree of commercial sugarcane varieties. These data will also be valuable for conservation and utilization of the genetic resources in germplasm collections.

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

INTRODUCTION

The accurate quantification of the genetic diversity of major agricultural crops is important both scientifically and socio-economically (Swanson, 1996). Concern has often been expressed that the practice of modern intensive plant breeding leads inevitably to a reduction in both diverse agricultural practices and genetic diversity of crops (Reeves et al., 1999). One way of averting this impending problem is to broaden the genetic base of the breeding program. A basic understanding of the genetic diversity that exists in the germplasm available for breeding is essential to the success of a breeding program.

Sugarcane is a large perennial grass mainly used for sugar and alcohol production worldwide, especially in tropical countries. The genus *Saccharum* L. is part of a polyploid complex within the Andropogoneae tribe of the grass family Poaceae and is composed of at least six distinct species *S. officinarum*, *S. barberi*, *S. sinensi*, *S. spontaneum*, *S. robustum*, and *S. edule* (Daniels and Roach, 1987; Nair et al., 1999). Modern sugarcane cultivars have a somatic chromosome number ranging from 100 to 130 derived from interspecific hybridization between *S. officinarum* L. ($2n$ = 70-140), which contributes to high sucrose content, and *S. spontaneum* L. ($2n$ = 36-128) for other desirable traits such as disease and pest resistance during a process called nobilization (Grivet and Arruda, 2002). Sugarcane cultivars grown in the world today share a limited genetic base because for most of the last century, sugarcane breeding activities were based on intercrossing the original nobilized clones and their derived progeny.

With the advent of molecular markers, it is now possible to make direct comparison of genetic diversity of sugarcane genotypes at the DNA level without some of the over simplifying assumptions associated with calculating genetic diversity based on pedigree history. Various molecular studies have been conducted to assess germplasm diversity within the genus *Saccharum*. Isozyme analyses (Glaszmann et al., 1989), as well as studies of ribosomal DNA (Glaszmann et al., 1990) and low-copy nuclear sequences (Lu et al., 1994), have been performed, and results reveal a limited variability within *S. officinarum*.

The present study examined the genetic diversity existing within the local collection of cultivars, elite lines and wild species *S. barberi* with random amplified polymorphic DNA (RAPD) fingerprinting. The use of RAPD primers offers the potential of acquiring more cost-effective data than in the case with other technologies. These primer sequences vary in different varieties of sugarcane, and this variability may be used to develop molecular markers for mapping sugarcane genes and traits, where these sequences are the part of sugarcane genome predicted to be most immediately useful to plant breeder and geneticists. Furthermore, this information will be useful for developing future breeding programs.

MATERIAL AND METHODS

Plant material

A total of 40 genotypes were evaluated (Table 1). These include experimental clones, commercial cultivars of *S. officinarum* and clones of *S. barberi*.

Table 1. Description of 40 genotypes used in genetic diversity study.

Serie No.	Genera and species	Clone name	Origin	Source of collection
Pop. 1	*Saccharum officinarum*	US-705	USA	AARI
Pop. 2		US-670	USA	AARI
Pop. 3		US-747	USA	AARI
Pop. 4		US-191	USA	AARI
Pop. 5		US-682	USA	AARI
Pop. 6		US-452	USA	AARI
Pop. 7		US-405	USA	AARI
Pop. 8		US-409	USA	AARI
Pop. 9		US-618	USA	AARI
Pop. 10		US-104	USA	AARI
Pop. 11		US-212	USA	AARI
Pop. 12		US-640	USA	AARI
Pop. 13		US-653	USA	AARI
Pop. 14		US-579	USA	AARI
Pop. 15		US-698	USA	AARI
Pop. 16		US-804	USA	AARI
Pop. 17		US-130	USA	AARI
Pop. 18		US-410	USA	AARI
Pop. 19		US-778	USA	AARI
Pop. 20		US-394	USA	AARI
Pop. 21		US-127	USA	AARI
Pop. 22		US-462	USA	AARI
Pop. 23		US-628	USA	AARI
Pop. 24		US-118	USA	AARI
Pop. 25		US-89	USA	AARI
Pop. 26		P-600	Unknown	UAF
Pop. 27		CP-81-1254	Canal point	UAF
Pop. 28		SP-302	Brazil	UAF
Pop. 29		SP-108	Brazil	UAF
Pop. 30		SP-646	Brazil	UAF
Pop. 31		CPF-85-1491	Canal point	UAF
Pop. 32		SPF-245	Brazil	UAF
Pop. 33		TCP-81-10	Brazil	UAF
Pop. 34		SP-722	Brazil	UAF
Pop. 35		CPF-247	Canal point	UAF
Pop. 36		CP-82-1172	Canal point	UAF
Pop. 37	*Saccharum barberi*	LCP-81	Canal point	UAF
Pop. 38		No. 51/77	Unknown	UAF
Pop. 39		No. 41/77	Unknown	UAF
Pop. 40		Katha	India	UAF

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

Selection of these genotypes was based on some important biometrical characteristics such as yield potential, maturity trend, ratoonability, salt tolerance, and disease (data not shown).

PCR assay for randomly amplified DNA fingerprinting

For DNA extraction, shoot apical meristems were selected as they contain low levels of polysaccharides and polyphenolic compounds. They were frozen, ground to a powder using liquid nitrogen, and then stored at -80°C. Genomic DNA was extracted following the CTAB method (Doyle and Doyle, 1987). RAPD primers were used to screen the genotypes to determine polymorphism levels, and the selected primers were scored across the population using the following protocols.

Polymerase chain reaction (PCR) conditions were optimized in a Gene Amp 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR for the amplification of

RAPD markers were carried out in a total volume of 25 µL containing 2.5 µL DNA templates, 2.0 µL primers, 4.0 µL dNTPs, 3.0 µL $MgCl_2$, 0.2 µL Taq polymerase, 8.3 µL d_3H_2O, 2.5 µL gelatin, and 2.5 µL 10X PCR buffer.

Reactions were run with the following cycling conditions: one cycle of denaturation for 5 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at the appropriate annealing temperature of 36°C and 2 min at 72°C for extension, and a final extension step of 5 min at 72°C at the end. The amplified products were mixed with an equal volume of loading dye and electrophoresed at 90 V on a 1.2% MetaPhor gel for approximately 2 h using 0.5X Tris-borate-EDTA (TBE) buffer along with a DNA molecular size marker. The gels were examined under a ultraviolet light transilluminator and photographed using the Syngene Gel Documentation System.

Scoring and analysis of RAPD data

Data were obtained from good-quality photographs of each amplification reaction. The bands were counted starting from the top of the lanes to the bottom. All visible and unambiguously scorable fragments amplified by the primers were scored under the heading of total scorable fragments. Amplification profiles of all the sugarcane plants were compared with each other and bands of DNA fragments were scored as present (1) or absent (0).

The data of the primers were used to estimate the genetic distances on the basis of the number of unshared amplified products. A dissimilarity matrix was generated using Nei's measures of genetic identity and genetic distance (Nei, 1978). In addition, population relationships were inferred using the unweighted pair group method with arithmetic mean (UPGMA) clustering method and the POPGENE software (version 3.5).

RESULTS

Primers used for RAPD and amplification

After optimization of the amplification conditions, DNA of 40 sugarcane accessions was amplified with 30 different decamer random primers, chosen randomly from the RAPD primer collection. All the primers were found to give reproducible bands (Table 2). A total of 238 DNA fragments were generated by the 30 primers with an average of about 7.93 bands per primer. Primers yielded bands ranging from 5 to 10.

Generally, the size and the number of bands produced were dependent on the nucleotide sequence of the primer used and the source of the template DNA. Reactions were duplicated to check the consistency of the amplified products. Only, easily resolved bright DNA bands were considered to be present and scored.

Polymorphism revealed by RAPD markers

Approximately 92.05% polymorphisms estimated from 219 of 238 fragments were polymorphic with 30 primers used among the 40 sugarcane accessions. The other 19 bands were monomorphic in the 40 accessions. In the present study, the 40 sugarcane accessions ap-

peared to show differences/variability with the 30 primers used. Although none of the primers individually was so informative as to differentiate all the accessions, highly polymorphic profiles were obtained with the 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.

Table 2. DNA polymorphism detected in sugarcane genotypes using Gene-Link decamer primers of A, B, C, D, G, and H series.

Serie No.	Primer name	Primer sequence (5'-3')	Size (bp)	Total No. of fragments generated	No. of polymorphic fragments	Percentage of polymorphic fragments
1	GLA-2	TGCCGAGCTG	200-550	9	8	88.89
2	GLA-5	AGGGGTCTTG	1000-2000	6	6	100.0
3	GLA-9	GGGTAACGCC	500-2000	9	9	100.0
4	GLA-15	TTCCGAACCC	500-1300	8	6	75.0
5	GLA-18	AGGTGACCGT	400-1500	9	8	88.89
6	GLB-3	CATCCCCCTG	200-600	6	6	100.0
7	GLB-5	TGCGCCCTTC	300-800	8	7	87.5
8	GLB-11	GTAGACCCGT	300-1200	7	7	100.0
9	GLB-17	AGGGAACGAG	100-500	7	7	100.0
10	GLC-2	GTCAGGCGTC	500-1200	8	7	87.5
11	GLC-5	GATGACCGCC	300-1200	9	7	77.79
12	GLC-7	GTCCCGACGA	150-500	9	8	100.0
13	GLC-9	CTCACCGTCC	500-1200	7	6	85.7
14	GLC-13	AAGCCTCGTC	600-1500	7	5	71.43
15	GLC-15	GACGGATCAG	400-1500	10	9	90.0
16	GLC-18	TGAGTGGGTG	350-700	8	8	100.0
17	GLD-1	ACCGCGAAGG	150-800	9	9	100.0
18	GLD-5	TGAGCGGACA	450-1500	10	10	100.0
19	GLD-7	TTGGCACGGG	450-1200	9	8	88.89
20	GLD-9	CTCTGGAGAC	500-1500	7	6	85.71
21	GLD-12	CACCGTATCC	600-1200	6	6	100.0
22	GLD-15	CATCCGTGCT	1000-2000	6	4	66.67
23	GLG-2	GGCACTGAGG	250-800	7	7	100.0
24	GLG-9	CTGACGTCAC	250-600	9	9	100.0
25	GLG-12	CAGCTCACGA	250-500	8	8	100.0
26	GLH-2	TCGGACGTGA	150-350	7	6	85.7
27	GLH-5	AGTCGTCCCC	350-650	9	9	100.0
28	GLH-15	AATGGCGCAG	700-1200	6	6	100.0
29	GLH-17	CACTCTCCTC	500-1500	10	8	80.0
30	GLH-20	GGGAGACATC	600-1600	9	9	100.0
Total	30	-	-	238	219	-
%	-	-	-	7.93	7.3	92.05

Genetics distances between the accessions

The genetic distance for RAPD data using 30 sugarcane accessions was constructed according to Nei (1978), as shown in Table 3a, b, c, and d, and relationships between accessions were presented graphically in the form of a dendrogram in Figure 1. The value of genetic distance ranging from 20.29 to 64.66% was observed among the 40 sugarcane accessions. The lowest genetic distance of 20.29 was seen in genotypes US-670 and US-191. These two genotypes differed from each other only in 20 bands with 13 different primers. The genotypes SP-108 and SP-646 were the second similar group with a genetic distance of 22.83%. The most dissimilar of all the accessions were US-394 and SPF-245 with a genetic distance of 64.66%.

Table 3. Nei's (1978) measures of genetic identity and genetic distance obtained for 40 sugarcane accessions using 10 RAPD markers

a.	1	2	3	4	5	6	7	8	9	10
1	****									
2	0.3655	****								
3	0.2719	0.3953	****							
4	0.3365	0.2029	0.3853	****						
5	0.3593	0.327	0.3853	0.2809	****					
6	0.3557	0.346	0.327	0.2809	0.3365	****				
7	0.346	0.263	0.3365	0.2719	0.327	0.3083	****			
8	0.3557	0.3083	0.3655	0.263	0.3365	0.3176	0.5242	****		
9	0.3557	0.327	0.4261	0.263	0.3557	0.2809	0.327	0.3557	****	
10	0.3176	0.346	0.3083	0.2809	0.263	0.2113	0.2719	0.2991	0.2991	****
11	0.3083	0.3953	0.3753	0.3083	0.346	0.346	0.3753	0.3655	0.3083	0.29
12	0.3655	0.4157	0.3365	0.3083	0.3083	0.29	0.263	0.3655	0.346	0.327
13	0.3356	0.327	0.327	0.2809	0.4157	0.263	0.29	0.2809	0.3753	0.3176
14	0.3557	0.3083	0.327	0.2991	0.3176	0.2991	0.3655	0.3176	0.3365	0.3365
15	0.3176	0.3655	0.3853	0.3557	0.3753	0.3557	0.4055	0.3557	0.3953	0.3953
16	0.3753	0.3853	0.3853	0.3176	0.3176	0.3753	0.3655	0.3365	0.4578	0.2991
17	0.3753	0.327	0.4261	0.3365	0.3365	0.3953	0.3655	0.4578	0.3753	0.3365
18	0.327	0.4157	0.3365	0.3083	0.327	0.3083	0.3753	0.3655	0.346	0.3083
19	0.3557	0.327	0.3853	0.2809	0.2455	0.263	0.3853	0.3557	0.3753	0.3176
20	0.3176	0.346	0.3655	0.2991	0.3953	0.2809	0.3853	0.3557	0.3176	0.3753
21	0.3365	0.3655	0.346	0.3557	0.2991	0.3365	0.3853	0.2991	0.4157	0.3176
22	0.3176	0.3853	0.3655	0.3176	0.3557	0.2991	0.4471	0.4366	0.4796	0.3953
23	0.3083	0.3365	0.2809	0.3083	0.4261	0.3083	0.3557	0.346	0.4471	0.29
24	0.3853	0.4796	0.3557	0.4055	0.4055	0.29	0.4578	0.4055	0.3853	0.346
25	0.3365	0.3853	0.3655	0.263	0.3176	0.263	0.3655	0.3176	0.3557	0.2809
26	0.4055	0.4366	0.4366	0.3655	0.4055	0.3083	0.3365	0.29	0.4261	0.346
27	0.3853	0.5245	0.3953	0.4686	0.3853	0.29	0.4157	0.4055	0.4686	0.327
28	0.3365	0.3655	0.327	0.263	0.3365	0.2991	0.3853	0.2809	0.3953	0.2809
29	0.3176	0.327	0.327	0.2809	0.3953	0.2991	0.3853	0.3365	0.4157	0.3953
30	0.3365	0.3655	0.4055	0.3557	0.3365	0.3557	0.3083	0.3365	0.4578	0.3365
31	0.3953	0.646	0.3655	0.263	0.3176	0.3365	0.327	0.3176	0.3753	0.3365
32	0.5716	0.5361	0.5131	0.5245	0.5018	0.4157	0.6084	0.4578	0.5478	0.4796
33	0.4796	0.29	0.4471	0.3365	0.4157	0.3365	0.4261	0.4578	0.4157	0.4366
34	0.5245	0.3853	0.4686	0.4157	0.4366	0.3753	0.4055	0.5245	0.5018	0.5018
35	0.4686	0.3953	0.4578	0.346	0.3655	0.3083	0.4157	0.4261	0.4261	0.4055
36	0.4796	0.4471	0.4686	0.4157	0.4157	0.3953	0.4686	0.3953	0.4578	0.3953
37	0.4471	0.4796	0.4157	0.4261	0.4686	0.4055	0.4796	0.4096	0.4906	0.4471
38	0.4157	0.3083	0.4471	0.3365	0.578	0.3557	0.4055	0.3753	0.4366	0.3953
39	0.4686	0.4366	0.4366	0.4261	0.4686	0.4261	0.4157	0.4471	0.4686	0.4261
40	0.4796	0.4471	0.4055	0.3953	0.4366	0.4366	0.4055	0.3753	0.5245	0.3953

b.	11	12	13	14	15	16	17	18	19	20
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	****									
12	0.3365	****								
13	0.327	0.3083	****							
14	0.327	0.3655	0.2809	****						
15	0.4471	0.4471	0.3557	0.2809	****					
16	0.3083	0.3655	0.4578	0.3365	0.4578	****				
17	0.3083	0.346	0.4157	0.3557	0.2991	0.3953	****			
18	0.263	0.3365	0.29	0.2542	0.346	0.346	0.327	****		
19	0.327	0.4055	0.2991	0.2991	0.3365	0.2809	0.3365	0.2368	****	

Continued on next page

Table 3. Continued.

b.	11	12	13	14	15	16	17	18	19	20
20	0.3853	0.346	0.3176	0.263	0.2809	0.3953	0.4366	0.327	0.2991	****
21	0.29	0.3083	0.3557	0.2991	0.3176	0.2455	0.3557	0.2719	0.3176	0.3753
22	0.4055	0.4471	0.3557	0.3365	0.3176	0.3176	0.4366	0.2719	0.2809	0.3365
23	0.3176	0.3753	0.3083	0.327	0.3655	0.29	0.346	0.2809	0.327	0.327
24	0.4796	0.4157	0.4686	0.4686	0.4471	0.3655	0.5837	0.4157	0.4055	0.3853
25	0.29	0.3083	0.3753	0.3365	0.3557	0.2991	0.3953	0.29	0.3176	0.3557
26	0.3953	0.4366	0.3853	0.3655	0.29	0.2719	0.4261	0.3176	0.327	0.3655
27	0.4578	0.3953	0.4261	0.4055	0.3655	0.4261	0.5361	0.3753	0.3853	0.3655
28	0.346	0.346	0.2991	0.3365	0.3557	0.3557	0.4366	0.327	0.3176	0.2991
29	0.346	0.346	0.263	0.2809	0.2809	0.3176	0.3753	0.3083	0.2809	0.3365
30	0.4055	0.3655	0.3953	0.3557	0.3557	0.2809	0.3365	0.3853	0.3365	0.3557
31	0.3655	0.3655	0.3753	0.3176	0.2809	0.2809	0.3365	0.346	0.2991	0.3176
32	0.3853	0.5361	0.5716	0.596	0.5245	0.4578	0.5245	0.4686	0.5245	0.6466
33	0.4471	0.4261	0.3753	0.3753	0.4366	0.4157	0.4157	0.3853	0.3176	0.3753
34	0.4261	0.4261	0.4366	0.4157	0.4157	0.5018	0.4366	0.3853	0.4578	0.4366
35	0.4157	0.3557	0.3655	0.3853	0.4055	0.4261	0.4261	0.3753	0.3083	0.4055
36	0.4686	0.4471	0.3753	0.4366	0.3953	0.4796	0.4366	0.4471	0.4366	0.5245
37	0.4796	0.4157	0.4055	0.4261	0.5361	0.4261	0.5837	0.5245	0.3853	0.4261
38	0.4471	0.4906	0.3365	0.4366	0.157	0.4157	0.5018	0.4055	0.3953	0.4796
39	0.4366	0.4157	0.346	0.4055	0.4261	0.4261	0.4471	0.3753	0.4055	0.4686
40	0.4471	0.3853	0.3753	0.3753	0.4578	0.3557	0.4157	0.4686	0.4157	0.3953

c.	21	22	23	24	25	26	27	28	29	30
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21	****									
22	0.3176	****								
23	0.2542	0.327	****							
24	0.4471	0.3655	0.4157	****						
25	0.2809	0.2809	0.29	0.29	****					
26	0.2719	0.327	0.3365	0.4157	0.3083	****				
27	0.3853	0.3655	0.3953	0.4366	0.3655	0.3176	****			
28	0.3557	0.2991	0.327	0.2719	0.1946	0.346	0.3853	****		
29	0.2455	0.2809	0.2542	0.3083	0.263	0.29	0.4055	0.3176	****	
30	0.2455	0.3953	0.3083	0.327	0.2809	0.3083	0.4261	0.3176	0.2283	****
31	0.263	0.2809	0.3083	0.3853	0.2455	0.327	0.3853	0.2991	0.263	0.2809
32	0.5018	0.5018	0.5131	0.4055	0.4366	0.4261	0.4471	0.4366	0.3953	0.4796
33	0.4157	0.3953	0.3853	0.4471	0.3953	0.4471	0.4055	0.4366	0.2991	0.4366
34	0.4578	0.3953	0.4906	0.5131	0.4796	0.4686	0.4086	0.5245	0.4578	0.4796
35	0.4686	0.4055	0.4578	0.3557	0.346	0.4157	0.4157	0.327	0.3655	0.4261

Continued on next page

Table 3. Continued.

c.	21	22	23	24	25	26	27	28	29	30
36	0.3753	0.5018	0.3853	0.5596	0.3953	0.346	0.3853	0.4796	0.4157	0.4366
37	0.4471	0.5596	0.4578	0.3953	0.4686	0.4157	0.3953	0.4261	0.346	0.4686
38	0.4157	0.4366	0.346	0.4055	0.3753	0.3853	0.5131	0.3753	0.3365	0.4157
39	0.346	0.4261	0.4366	0.4366	0.4471	0.4366	0.5018	0.4261	0.4055	0.3853
40	0.3557	0.4366	0.29	0.4686	0.3365	0.4261	0.5131	0.3365	0.3176	0.3753

d.	31	32	33	34	35	36	37	38	39	40
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31	****									
32	0.4578	****								
33	0.3753	0.5245	****							
34	0.5018	0.5716	0.3176	****						
35	0.327	0.5906	0.3083	0.3655	****					
36	0.4366	0.5018	0.3753	0.4578	0.4471	****				
37	0.4261	0.4906	0.346	0.4906	0.3953	0.3655	****			
38	0.4578	0.4578	0.2991	0.3365	0.3655	0.3953	0.4055	****		
39	0.4906	0.5361	0.3655	0.3655	0.4366	0.4055	0.5245	0.4261	****	
40	0.3365	0.5478	0.3953	0.4578	0.4055	0.4366	0.4055	0.3753	0.4471	****

Clustering pattern

The cluster analysis based on dissimilarity values classified all the sugarcane accessions into three major groups I, II and III (Figure 1) while SPF-245 forms an independent cluster. Clustering of different genotypes into strictly separate groups was not readily apparent in group I due to very low genetic distance between the genotypes. However, this group was

resolved into three distinct subgroups, A, B and C. Subgroup A comprises 2 genotypes belonging to the USA. A second subgroup, B, consists of 17 genotypes of which 11 originated in the USA (US-579, US-394, US-698, US-410, US-778, US-462, US-804, US-127, US-628, US-118, and US-89), three originated in Brazil (SP-108, SP-646 and SP-302; SP stands for São Paulo), CPF-85-1491 originated at Canal Point, and Katha cane is of Indian origin. The origin of genotype P-600 was not known. A third subgroup comprises 12 genotypes, all of them having the USA as place of origin (US-670, US-191, US-405, US-409, US-653, US-452, US-104, US-618, US-682, US-640, US-212, and US-130). It is clear from the above mentioned results that most of the genotypes that form group I belong to USA. Genotypes constituting groups II and III have more diverse origin than those falling in group I.

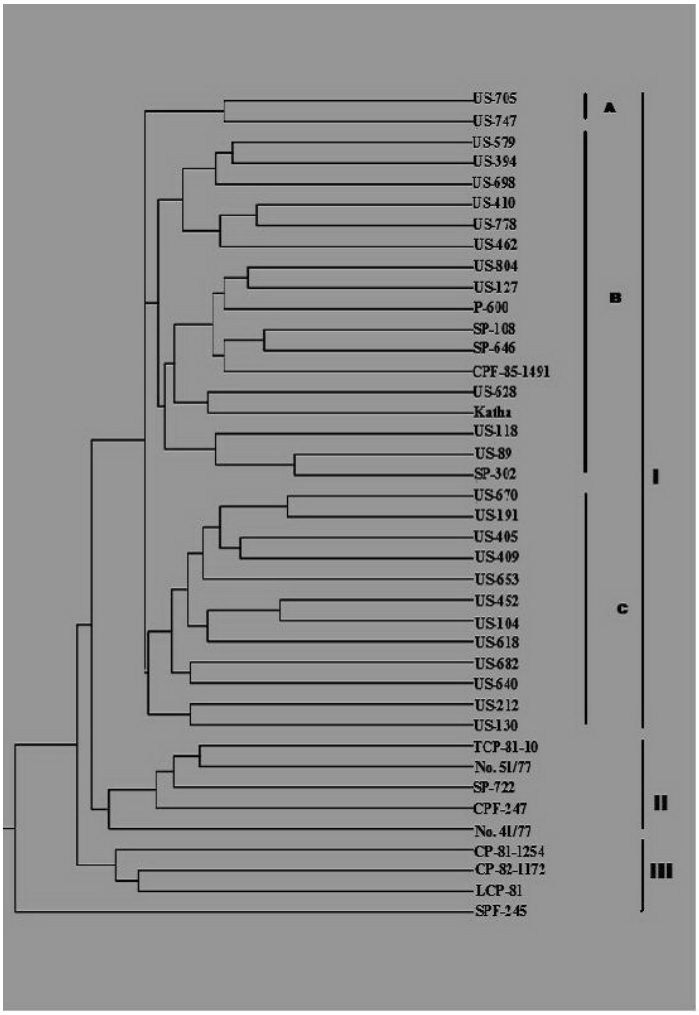

Figure 1. Dendrogram of 40 sugarcane accessions developed from RAPD data using unweighted pair group method with arithmetic mean (UPGMA) based on Nei's (1978) genetic distance.

Group II includes five genotypes, of which two genotypes No. 51/77 and No. 41/77 belong to *S. barberi*, while the other three belong to *S. officinarum* (TCP-81-10, SP-722 and CPF-247). TCP-81-10 and SP-722 originated in Brazil and CPF-247 originated at Canal Point but selection was carried out from seed at Faisalabad.

Three genotypes namely CP-81-1254, CP-82-1172 and LCP-81 originated at Canal Point. CP-81-1254 and CP-82-1172 belong to *S. officinarum*, and LCP-81 belongs to *S. barberi* obtained in a selection of Louisiana (L) with seeds from Canal Point. *S. barberi* genotypes do not form a distinct cluster but cluster with other genotypes belonging to *S. officinarum*, which indicate genetic similarities existing between them.

DISCUSSION

A basic understanding of the genetic diversity that exists in the germplasm available for breeding is essential to the success of a breeding program. This knowledge is useful in the utilization and management of genotypes and indeed genes in the breeding gene pool.

In sugarcane, crosses are planned between genotypes from divergent backgrounds to maximize heterosis while increasing genetic diversity in the gene pool. Sugarcane breeders have traditionally relied on pedigree records when planning divergent crosses. Faulty genealogy and inadvertent mislabeling of clones adversely complicate genetic diversity estimates that rely solely on pedigree history. Sugarcane breeders are notorious for crossing mostly parents that have attained the so-called proven cross status. That is, those parents that produce elite progenies are retained for further crossing to the detriment of newer parents as evident from the high number of vintage clones still involved in the parentage of newer cultivars (Deren, 1995). Potential parents are selected largely based on their performance as clones in advanced stage trials. Therefore, continuous selections for the same traits narrow genetic diversity to the extent that it is difficult to predict diversity based on pedigree history alone. With the advent of molecular markers, it is now possible to make direct comparison of genetic diversity at the DNA level without some of the over simplifying assumptions associated with calculating genetic diversity based on pedigree history (Brar, 2002).

Rapid advances in the field of molecular biology and its allied sciences made the use of molecular markers a routine practice providing plant breeders a precise tool in analyzing genetic diversity for plant improvement. For achieving improved productivity in sugarcane crop, it is essential to maintain a high degree of genetic diversity among the commercial varieties and breeding populations. Though diversification is apparent in the current germplasm collection, an assessment of its genetic diversity is lacking.

Therefore, the present investigation reported 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. Using RAPD as genetic markers, as high as 92.05% polymorphic bands were detected in 40 accessions of sugarcane; similar studies were conducted by Burner et al. (1997) and Nair et al. (2002).

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 0.20 to 0.65 with an average of 0.42 suggested that the level of genetic diversity among the sugarcane accessions is moderate. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed genetic diversity as well (Arceneaux, 1967; Harvey and Botha, 1996).

In the present study, four *S. barberi* clones were used, which instead of forming distinct/separate clusters formed clusters with *S. officinarum* clones, 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*, were the progenitors of modern sugarcane, where *S. officinarum* may be derived from *S. robustum*, and that *S. barberi* and *S. sinense* were cultivated forms of interspecific hybrids between *S. spontaneum* and *S. officinarum.* The above hypothesis is confirmed by the present study, as four *S. barberi* clones namely LCP-81, No. 51/77, No. 41/77, and Katha fall in different clusters.

LCP-81 clusters with CP-82-1172 and CP-81-1172, both of which are cultivated varieties of *S. officinarum*. CP-82-1172 clusters with LCP-81 at a genetic distance of 0.36 and CP-81-1172 clusters with LCP-81 at a genetic distance of 0.39 because CP-82-1172 is closer to LCP-81 as compared to CP-81-1172. Furthermore, both of them have the same origin, namely Canal Point, and share many bands in common with each other and LCP-81.

The *S. barberi* clones No. 51/77 and No. 41/77 fall in the same cluster II, which also consists of three *S. officinarum* varieties, namely CPF-247, SP-722 and TCP-81-10. These three accessions lie between the two *S. barberi* clones. Genetic distances between clone No. 51/77 and CPF-247, SP-722 and TCP-81-10 are 0.36, 0.33 and 0.29, respectively. While genetic distances between clone No. 41/77 and CPF-247, SP-722 and TCP-81-10 are 0.43, 0.36 and 0.36, respectively.

The fourth clone, named Katha and of Indian origin, falls in cluster I, which also consists of genotypes that belong to *S. officinarum*. Thus, the *S. officinarum* varieties and *S. barberi* clones have genetic distances in the range of 29 to 54%, showing that the level of genetic diversity between these sugarcane accessions is low. This range is similar to that obtained by Pan et al., 2005 (60.5 to 88.5%) and narrower than that reported for North American and old world *Saccharum* (36 to 76%; Burner et al., 1997). SPF-245 is the most distinct accession forming a separate cluster, and the highest genetic distance, 64.66%, exists between SPF-245 and US-394, both belonging to *S. officinarum.*

Thus, mean genetic distance among the 40 accessions in this study was 42%, implying that the genetic diversity among the genotypes is limited. This probably arises from the lack of parental diversity, with few clones being 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 (Sreenivasan et al., 1987).

In this study, genetic analysis was performed using RAPD markers because this method does not require knowledge of the sequence of the DNA under study (Wolfe and Liston, 1998). Primers were designed on a random basis, with the sole constraint being guanine-cytosine content. It permitted simultaneous examination of multiple loci in a single PCR. The same panel of primers can be used to study any organism. The most attractive feature of RAPD analysis is that it can be used on pooled DNA samples to rapidly screen for linked DNA markers (Michelmore et al., 1991).

Like all other techniques, the RAPD-PCR has limitations, among which are the complexity of resultant fingerprint patterns and the fact that heterozygotes cannot be distinguished from homozygotes (Thormann et al., 1994; Pillay and Kenny, 1995). Another shortcoming

of RAPD is its low band repeatability and occurrence of pseudobands. The concern about reproducibility of RAPD markers, however, was overcome through choosing an appropriate DNA extraction protocol to remove any contaminants (Micheli et al., 1994), by optimizing the parameters used (Ellsworth et al., 1993; Skroch and Nienhuis, 1995), by testing several oligonucleotide primers and scoring only the reproducible DNA fragments (Kresovich et al., 1992; Yang and Quiros, 1993), and by using an appropriate DNA polymerase band.

This study will facilitate the use of RAPD-PCR fingerprints in marker-assisted applications in sugarcane breeding. First, in this study primers were identified that generate substantial polymorphisms among *S. barberi* and elite sugarcane germplasm. Similar genetic analysis is also applicable to other sugarcane-related wild species, such as *S. sinense* (Brandes, 1958), *S. robustum* (Grassl, 1977), *S. edule* (Daniels and Roach, 1987), etc. Second, given the resource limitations on conserving clonal germplasm collections, the study demonstrated an approach for identifying and maintaining diverse clones in *S. officinarum* and *S. barberi* core collections (Glaszmann et al., 1989; Sobral and Honeycut, 1993; Lu et al., 1994). Third, the study demonstrates the potential of specific RAPD-PCR markers for identifying *S. barberi* clones and elite cultivars.

The information derived from the present study may be helpful for sugarcane breeders in planning future breeding programs for the development of high-yielding sugarcane varieties in Pakistan.

REFERENCES

Arceneaux G (1967). Cultivated sugarcane of the world and their botanical derivation. *Proc. Int. Soc. Sugar Cane Technol.* 12: 844-845.

Brandes EW (1958). Origin, Classification and Characteristics. In: Sugarcane (*Saccharum officinarum* L.) (Artschwager E and Brandes EW, eds.). U.S. Department of Agriculture Handbook, Washington D.C., 1-35.

Brar DS (2002). Molecular Marker Assisted Breeding. In: Molecular Techniques in Crop Improvement (Jain SM, Brar DS and Ahloowalia BS, eds.). Kluwer Academic Publishers, Netherlands, 55-83.

Burner DM, Pan YB and Webster RD (1997). Genetic diversity of North American and Old World *Saccharum* assessed by RAPD analysis. *Genet. Res. Crop Evol.* 44: 235-240.

Daniels J and Roach BT (1987). Taxonomy and Evolution in Sugarcane. In: Sugarcane Improvement Through Breeding (Heinz DJ, ed.). Elsevier Press, Amsterdam, 7-84.

Deren CW (1995). Genetic base of U.S. mainland sugarcane. *Crop Sci.* 35: 1195-1199.

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19: 11-15.

Ellsworth DL, Rittenhouse KD and Honeycutt RL (1993). Artifactual variation in randomly amplified polymorphic DNA banding patterns. *Biotechniques* 14: 214-217.

Glaszmann JC, Fautret A, Noyer JL, Feldmann P, et al. (1989). Biochemical genetic markers in sugarcane. *Theor. Appl. Genet.* 78: 537-543.

Glaszmann JC, Lu YK and Lanaud C (1990). Variation of nuclear ribosomal DNA in sugarcane. *J. Genet. Breed.* 44: 191-198.

Grassl CO (1977). The origin of the sugar producing cultivars of *Saccharum*. *Sugarcane Breed. Newsl.* 39: 8-33.

Grivet L and Arruda P (2002). Sugarcane genomics: depicting the complex genome of an important tropical crop. *Curr. Opin. Plant Biol.* 5: 122-127.

Harvey M and Botha FC (1996). Use of PCR-based methodologies for the determination of DNA diversity between *Saccharum* varieties. *Euphytica* 89: 257-265.

Kresovich S, Williams JGK, McFerson JR, Routman EJ, et al. (1992). Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. *Theor. Appl. Genet.* 85: 190-196.

Lu YH, D'Hont A, Walker DIT, Rao PS, et al. (1994). Relationships among ancestral species of sugarcane revealed with RFLP using single copy maize nuclear probes. *Euphytica* 78: 7-18.

Micheli MR, Bova R, Pascale E and D'Ambrosio E (1994). Reproducible DNA fingerprinting with the random amplified

polymorphic DNA (RAPD) method. *Nucleic Acids Res.* 22: 1921-1922.
Michelmore RW, Paran I and Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. U. S. A.* 88: 9828-9832.
Nair NV, Nair S, Sreenivasan TV and Mohan M (1999). Analysis of genetic diversity and phylogeny in *Saccharum* and related genera using RAPD markers. *Genet. Res. Crop Evol.* 46: 73-79.
Nair NV, Selvi A, Sreenivasan TV and Pushpalatha KN (2002). Molecular diversity in Indian sugarcane cultivars as revealed by randomly amplified DNA polymorphisms. *Euphytica* 127: 219-225.
Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
Pan YB, Miller J, Schnell R, Richard E, et al. (2003). Application of Microsatellite and RAPD Fingerprints in the Florida Sugarcane Variety Program. In: Plant Animal Genome VX Conference, Houma, Abstract 43.
Pan YB, Burner DM, Legendre BL, Grisham MP, et al. (2005). An assessment of the genetic diversity within a collection of *Saccharum spontaneum* L. with RAPD-PCR. *Genet. Res. Crop Evol.* 51: 895-903.
Pillay M and Kenny ST (1995). Anomalies in direct pair-wise comparisons of RAPD fragments for genetic analysis. *Biotechniques* 19: 694-6, 698.
Reeves J, Law J, Donin P, Koebner MR, et al. (1999). Changes over time in the genetic diversity of UK cereal crops. Available at National Institute of Agricultural Botany [http://apps3.fao.org/wiews/Prague/Paper12.htm], Cambridge.
Skroch P and Nienhuis J (1995). Impact of scoring error and reproducibility RAPD data on RAPD based estimates of genetic distance. *Theor. Appl. Genet.* 91: 1086-1091.
Sobral BWS and Honeycut RJ (1993). High output genetic mapping of polyploids using PCR-generated markers. *Theor. Appl. Genet.* 86: 105-112.
Sreenivasan TV, Ahloowalia BS and Heinz DJ (1987). Cytogenetics. In: Sugarcane Improvement Through Breeding (Heinz DJ, ed.). Elsevier, Amsterdam, 211-253.
Swanson T (1996). Global values of biological diversity: the public interest in the conservation of plant genetic resources for agriculture. *Plant Genet. Res. Newsl.* 105: 1-7.
Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, et al. (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. *Theor. Appl. Genet.* 88: 973-980.
Weir BS (1990). Genetic Data Analysis: Methods for Discrete Population Genetic Data. Sinauer Associates Inc., Sunderland.
Wolfe AD and Liston A (1998). Contribution of PCR-based Methods to Plant Systematics and Evolutionary Biology. In: Molecular Systematics of Plants II: DNA Sequencing (Soltis DE, Soltis PS and Doyle JJ, eds.). Kluwer, Boston, 43-86.
Yang X and Quiros C (1993). Identification and classification of celery cultivars with RAPD markers. *Theor. Appl. Genet.* 86: 205-212.