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ABSTRACT

This experiment was conducted to reveal genetic diversity among 38 genotypes of sugarcane (*Saccharum officinarum* L.) using RAPD markers. The population consisted of 8 genotypes from Australia, 7 from Africa, 10 from America, and 13 from Asia. Genetic similarity was ranging from 17% to 97% , with the average of 57%. UPGMA dendrograms divided the population into three major groups i.e. group 1, 2, and 3 which consisted of 23, 10, and 5 genotypes, respectively. Each major group comprised genotypes of different geographical origins. The dendrogram divided each group into some subgroups. There were 8 subgroups i.e. 4 subgroups in group 1, 2 subgroups in group 2, and 2 subgroups in group 3. Some genotypes of same geographical origin were clustered into in at least 3 different subgroups, meaning that they were genetically dissimilar. On the other hand, some other genotypes of different geographical origin were clustered into the same subgroup, meaning that they were genetically similar. This data would help sugarcane breeders to select parents for hybridization in order to maximize heterosis. This could be conducted by selecting parents of dissimilar genotypes.

Keywords: diversity; RAPD; sugarcane

INTRODUCTION

Modern sugarcane cultivars were originated from interspecific hybridization of *Saccharum officinarum* L. (2n = 80), which was superior in sugar content, and *Saccharum spontaneum* L. (2n = 40-128), and it was superior in other characters such as tolerant to some biotic and abiotic stresses. This interspecific hybridization to improve *Saccharum officinarum* characters is well known as nobilization. The hybrids derived from the nobilization were then used to develop new clones with more desirable characters. This demonstrated that sugarcane breeding program had basically been conducted using genetically narrow germplasms, leading to a relatively slow breeding progress. This narrow genetic base of sugarcane as represented by low average genetic distance between genotypes had been reported by some researchers as follows: 29% (Nair *et al.*, 2002), 39 % (Khan *et al.*, 2009), 13% (Kawar *et al.*, 2009), 42% (Tabasum *et al.*, 2010), 17% (Govindaraj *et al.*, 2011), 49% (Devarumath *et al.*, 2012), and 28% (Saravanakumar *et al.*, 2014). This reports demostrated that a large part of the genome was similar among sugarcane genotypes under study.

One way of enhancing breeding progress was using parents of genetically distant genotypes so that chances of getting heterosis and obtaining superior progenies with different favourable alleles was greater. Nair (2011) described sugarcane verietal development in India that had been carried out mostly by bi-parental hybridization. Parental selection was carried out not only on the basis of the pheno-typic performance with respect to yield, quality, adaptability and disease resistance, but also on the basis of genetic diversity between parents. Therefore, it was necessary to asses genetic diversity of sugarcane germplasm collection before being used as parents in hybridization.

Genetic diversity in a plant population could be assessed using morphological, biochemical, and molecular markers. While morphological and biochemical markers were influenced by environmental factors, meanwhile, molecular markers were not. Therefore, the use of molecular marker lead to more reliable results in genetic diversity assessment. Molecular markers had been used to study genetic diversity of

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ious plants, one of them was RAPD (randomly amplified polymorphic DNA) marker. RAPD markers had been used to study genetic diversity of various plants such as mango (Samal *et al*., 2012), Safflower (Amini *et al*., 2008), banana (Santos *et al*., 2010), *Capsicum* sp. (Thul *et al*., 2012), Jerusalem artichoke (Wangsomnuk *et al*., 2011), piper (Sen *et al*., 2010), *Persea bombycina* (Bhau *et al*., 2009), basil (Chen *et al*., 2013), rice (Arshad, *et al*., 2011), Hevea (Lam *et al*., 2009), *Carica cubensis* (Rodriguez *et al*., 2010), soybean (Al-Saghir and Salam, 2011), cowpea (Anatala *et al*., 2014) and physic nut (Rafii *et al*., 2012).

This research was conducted to study genetic diversity of sugarcane population using RAPD markers. This genetic diversity data, together with the phenotypic data, would be expectedly useful for selecting parents in a sugarcane breeding program to maximize heterosis effects.

MATERIALS AND METHODS

Genetic materials of sugarcane were generously provided by Gunung Madu Plantations Company, Terbanggi Besar, Lampung Province, Indonesia. This experiment was conducted from February-December 2013. Thirty eight accessions of sugarcane genotypes from Australia, America, Asia, and Africa were used in this study (Table 1). One-node cuttings were planted in a mixture of soil and compost (1:1 v/v) contained in a polybag and maintained for 4 months. Watering was routinely done to allow the buds to develop shoots and in turn the shoots to produce roots. The main stems were then cut off to allow suckers to grow. One month later, the suckers were ready for DNA extraction.

Young leaf rolls of young suckers (about 3 cm in length) were used as source of DNA. Suckers were harvested and collected in an iced box and brought to the laboratorium. Suckers were washed under running tap water and one outer layer of leaves were peeled. Leaf rolls were made by transversally cutting the suckers into disks of about 5 mm thick. Approximately 1 g of leafrolls was soaked in cold absolute ethanol contained in mortar for 30 minutes. The ethanol was decanted and allowed to evaporate and then 10 ml of freshly-made homogenization buffer + 0.2 g polyvinil pyrolidone (PVP) were put into the sample. The homogenization buffer was comprised of 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2 M NaCl, and 2% CTAB (Vaze *et al*., 2010). The samples were then quickly ground with mortars and pestles and poured into 50- ml tubes.

Table 1. Sugarcane genotypes and their origins for use in genetic diversity study

No.	Genotypes	Origin	No.	Genotypes	Origin
1	AUS-2	Australia	21	CP-51-21	America
2	AUS-3	Australia	22	Mex-69-1460	America
3	AUS-5	Australia	23	H-58-4748	America
4	AUS-8	Australia	24	SP-72-6163	America
5	2053-BEST	Australia	25	HJ-57-41	America
6	Q-96	Australia	26	PSGM-92-2290	Asia
7	Q-100	Australia	27	BL-666	Asia
8	Q-190	Australia	28	PSGM-92-2075	Asia
9	M-4	Africa	29	BW-3605	Asia
10	N-55-805	Africa	30	PHIL-71-15	Asia
11	N-55-1164	Africa	31	F-01	Asia
12	N-56-42	Africa	32	MT-72	Asia
13	M-442-51	Africa	33	TC-15	Asia
14	R-570	Africa	34	842388	Asia
15	R-579	Africa	35	GP-06	Asia
16	PR-980	America	36	BO-645	Asia
17	SP-79-2278	America	37	R3-PPB-X2	Asia
18	Irv-93-1030	America	38	SS-83	Asia
19	Irv-93-770	America			
20	H-57-5174	America			

After being added with 1% β-mercapto-ethanol, the mixture was quickly spinned and incubated at 65°C for 60 minutes, allowed to cool down until room temperature, added with cloroform and isoamylalcohol (24:1), and centrifuged at 6000 rpm at 4° C for 20 minutes. The supernatant in the middle layer was taken up using micropipets and put into 15 ml tubes. One-fifth volume of NaCl 5 M and one volume of cold propanol were added and the mixture was incubated overnight at -20° C. The mixture was then centrifuged at 6000 rpm at 4° C for 20 minutes. The supernatant was poured away and the pellet was washed with 500 μl of 70% ethanol by spinning at 6000 rpm at 4° C for 20 minutes. The pellet was then air-dried and added with 60 μl of TE buffer.

DNA quality was checked using A260/A280 ratio, which indicated DNA absorbance at 260 nm divided by DNA absorbance at 280 nm using scanning UV/visible spectrophotometer (Unico SQ-2800 Single Beam, United Products and Instruments). If the A260/A280 ratio of the DNA was 1.8-2.0, the DNA was considered to be of high purity. In addition, DNA quality was also checked using electroproresis to know whether the genomic DNA was intact or fragmented. Electrophoresis was done using TBE buffer at 90 V on 1% agarose gel for 120 minutes. Bands were visualized with MultiDoc-It™ Imaging System (Ultra-Violet Products Ltd., UK) connected to a computer.

Polymerase chain reaction (PCR) was conducted in a thermocycler (Techne-5000, Bibby Scientific, UK). The machine was programmed at heated lid of 105° C, the preheated lid was on, the pause was off, and the predenaturation was set at temperature of 95° C for 4 minutes followed 35 cycles of reactions consisting of denaturation at 95° C for 30 seconds, annealing at 37° C for 60 seconds, and extension at 72° C for 120 seconds. Final extension was set at 72° C for 7 minutes and the reaction was stopped with final hold at 10° C. PCR was done in 25 μl reaction mixture contained in 200-μl tubes. The reaction mixture consisted of 1 μl template DNA 300 ng μl^{-1}, 2 μl primer 10 μM, 12.5 μl FastStart PCR Master (Roche Life Science, Switzerland), and 9,5 μl H_2O. Amplicons mixed with a loading dye and a DNA molecular size marker were electrophoresed and visualized as previously described.

Clear, unambiguous, and reproducible bands of amplified products generated by electrophoresis were scored as 1 (present) and 0 (absent). The data were then used to make a similarity matrix according to Nei's measures of genetic identity and genetic distance (Nei, 1978). Based on the matrix, a dendrogram showing clusters among genotypes within population was made using the UPGMA (unweighted pair group with arithmatic mean) method.

RESULTS AND DISCUSSION

Twenty decamer primer were selected from 30 random primers used by Tabasum *et al.* (2010) to study genetic diversity of sugarcane. After PCR condition had been optimized, the 20 decamer primers were screened and 5 primers that resulted in clear, unambiguous, reproducible, and polymorphic DNA bands were selected. Those selected primers were then used to generate bands in PCR reaction using DNA template of 38 sugarcane genotypes. A total of 35 bands were generated with an average of 7 bands per primer were produced ranging from 4 to 9 bands per primer (Table 2).

The average percentage of polymorphic bands per primer was 78.45% ranging from 50-100% (Table 2). Band size was ranging from 200-10000 bp and mostly in the range of 200-400 bp. The highest number of polymorphic bands was produced by primer GLB-17 (9 bands) and the lowest by GLG-12 (2 bands). DNA profile generated by electrophoresis of PCR products using primer GLA-2 was shown in Figure 1.

Based on the DNA profile resulted from electrophoresis of PCR products, a table depicting genetic similarity between genotypes was constructed using calculation as described by Nei (1978) (Table 3). Genetic similarity was ranging from 17% to 97%, the lowest being between 842388 and BL-666 and between Irv-93-1030 and BL-666 and the highest between SP-79-2278 and PSGM-92-2075 and between Q-100 and HJ-57-41 (Table 3). The average genetic similarity between genotypes in the population was 57% (genetic distance of 43%).

Table 2. Decamer primers used to study genetic similarity among 38 sugarcane accessions

No.	Name of primers	Sequence of primers (5'-3')	Number of bands	Number of polymorphic bands	% Polymorphic bands	Band size range (bp)
1	GLG-12	5'CAGCTCACGA3'	4	2	50	200-400
2	GLC-2	5'GTCAGGCGTC3'	9	8	88.8	250-750
3	GLA-2	5'GGGTAACGCC3'	9	7	77.78	300-1000
4	GLB-17	5'AGGGAACGAG3'	9	9	100	250-10000
5	GLC-15	5'GACGGATCAG3'	4	3	75	300--800
	Total		35	29		
	Average		7	5.8	78.45	

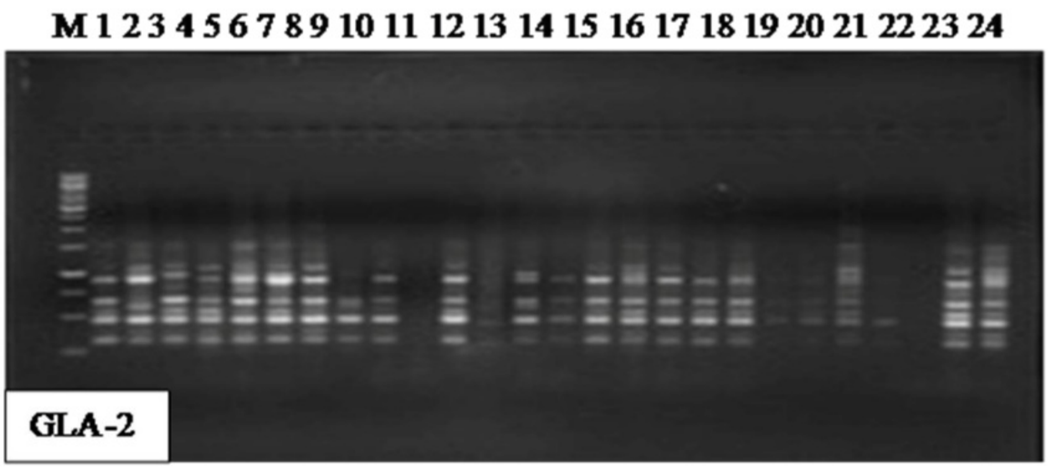

Figure 1. Banding pattern of amplified product of polymerase chain reaction (PCR) of DNA isolated from different sugarcane accessions. The primer used in the PCR was GLA-2. M= 1-Kb ladder. 1-24 = DNA samples.

Table 3. Genetic similarity of 38 sugarcane genotypes calculated according to Nei (1978) using polymorphic RAPD markers

No.	Genotipe	PSGM-92-2290	BL-666	PSGM-92-2075	BW-3605	PHIL-71-15	F-01	MT-72	TC-15	842388	GP-06	BO-645	R3-PPB-X2	SS-83
1	PSGM-92-2290	1												
2	BL-666	0.48570	1											
3	PSGM-92-2075	0.77420	0.5428	1										
4	BW-3605	0.57140	0.6857	0.5714	1									
5	PHIL-71-15	0.48570	0.7142	0.5428	0.7428	1								
6	F-01	0.57140	0.5142	0.4571	0.4857	0.5142	1							
7	MT-72	0.68570	0.5142	0.6285	0.3714	0.5142	0.7714	1						
8	TC-15	0.51420	0.4571	0.5142	0.5428	0.4000	0.3714	0.4285	1					
9	842388	0.57142	0.1714	0.4000	0.3714	0.2457	0.5428	0.4857	0.7142	1				
10	GP-06	0.51428	0.6857	0.5714	0.7142	0.7428	0.4285	0.4285	0.6000	0.4285	1			
11	BO-645	0.42857	0.8285	0.4857	0.8000	0.7142	0.5714	0.4571	0.5714	0.2857	0.7428	1		
12	R3-PPB-X2	0.57142	0.8000	0.5714	0.6571	0.6285	0.6571	0.6000	0.3714	0.3142	0.5428	0.6285	1	
13	SS-83	0.31428	0.4857	0.3142	0.6285	0.6000	0.3428	0.2285	0.7428	0.5714	0.7428	0.6571	0.3428	1
14	PR-980	0.71428	0.5485	0.8285	0.5142	0.5428	0.5142	0.6285	0.5142	0.4000	0.5142	0.4857	0.5714	0.3714
15	SP-79-2278	0.80000	0.5714	0.9714	0.5428	0.5142	0.4857	0.6571	0.5428	0.4285	0.5428	0.5142	0.6000	0.2857
16	Irv-93-1030	0.68571	0.1714	0.6285	0.3714	0.2857	0.4285	0.5428	0.6000	0.7714	0.3714	0.2285	0.3142	0.4571
17	H-57-5174	0.74285	0.5142	0.8000	0.5428	0.6285	0.6571	0.7142	0.4857	0.4857	0.6000	0.5142	0.6000	0.3428
18	CP-51-21	0.85714	0.5714	0.7428	0.4857	0.5714	0.5428	0.7142	0.5428	0.4285	0.4857	0.4571	0.5428	0.2857
19	Mex-69-1460	0.71428	0.5428	0.7142	0.6857	0.6571	0.6285	0.6285	0.5714	0.4571	0.5714	0.6000	0.6285	0.4285
20	H-58-4748	0.71428	0.6000	0.7142	0.5142	0.3714	0.6285	0.6857	0.4000	0.4000	0.4000	0.4857	0.6285	0.2571
21	SP-72-6163	0.65710	0.4285	0.6000	0.4000	0.4285	0.8000	0.8571	0.4000	0.5142	0.4000	0.4285	0.6285	0.2571
22	HJ-57-41	0.74280	0.5714	0.6857	0.6000	0.6285	0.6000	0.6000	0.4857	0.4857	0.5428	0.5142	0.6000	0.4000
23	Irv-93-770	0.48571	0.2000	0.4285	0.4000	0.3142	0.4571	0.4000	0.5714	0.8000	0.4000	0.3142	0.3428	0.6000
24	M-4	0.77140	0.5428	0.6000	0.6285	0.6571	0.6857	0.6857	0.5714	0.5142	0.5714	0.5428	0.6285	0.4285
25	N-55-805	0.54280	0.6000	0.5428	0.4571	0.4285	0.8571	0.7428	0.5142	0.5142	0.4571	0.6000	0.6857	0.3142
26	N-55-1164	0.42850	0.7714	0.4857	0.8000	0.7714	0.5142	0.4000	0.5142	0.2857	0.8000	0.8857	0.5714	0.6571
27	N-56-42	0.62850	0.5714	0.5714	0.4857	0.4571	0.8285	0.7714	0.4285	0.4857	0.3714	0.5142	0.7142	0.2285
28	M-442-51	0.77140	0.4857	0.7142	0.5714	0.6000	0.6285	0.6857	0.4571	0.4571	0.4571	0.4857	0.6285	0.3714
29	R-570	0.34280	0.7428	0.3428	0.7142	0.7428	0.5428	0.4285	0.4857	0.3142	0.7142	0.8000	0.6000	0.6285
30	R-579	0.40000	0.7428	0.4571	0.8285	0.8000	0.4285	0.3142	0.5428	0.3142	0.8285	0.8571	0.5428	0.7428
31	AUS-2	0.57142	0.6285	0.4571	0.4285	0.5142	0.8857	0.7714	0.4285	0.4857	0.4285	0.5714	0.6571	0.2857
32	AUS-3	0.51428	0.6857	0.4571	0.4857	0.5142	0.8285	0.7714	0.4857	0.4285	0.4857	0.6285	0.7142	0.3428
33	AUS-5	0.85714	0.4571	0.8000	0.4857	0.5142	0.6000	0.7714	0.4285	0.4857	0.4285	0.4000	0.6000	0.2857
34	AUS-8	0.62850	0.6857	0.6857	0.5428	0.5142	0.6000	0.6000	0.3714	0.3714	0.4857	0.5142	0.7714	0.2857
35	2053-BEST	0.74280	0.5714	0.6857	0.6000	0.6285	0.6000	0.6571	0.5428	0.4857	0.6000	0.5142	0.6000	0.4000
36	Q-96	0.68570	0.6857	0.4571	0.5428	0.5142	0.6571	0.6000	0.3714	0.3714	0.4857	0.5714	0.7142	0.3428
37	Q-100	0.71420	0.5428	0.6571	0.6285	0.6571	0.6285	0.5714	0.4571	0.4571	0.5142	0.5428	0.5714	0.4285
38	Q-190	0.45710	0.7428	0.5142	0.7142	0.6857	0.7142	0.6000	0.4857	0.3714	0.6000	0.8571	0.7142	0.5714

Table 3. Genetic similarity of 38 sugarcane genotypes calculated according to Nei (1978) using polymorphic RAPD markers (continued)

No.	Genotipe	PR-980	SP-79-2278	Irv-93-1030	H-57-5174	CP-51-21	Mex-69-1460	H-58-4748	SP-72-6163	HJ-57-41	Irv-93-770	M-4	N-55-805	N-55-1164
1	PSGM-92-2290													
2	BL-666													
3	PSGM-92-2075													
4	BW-3605													
5	PHIL-71-15													
6	F-01													
7	MT-72													
8	TC-15													
9	842388													
10	GP-06													
11	BO-645													
12	R3-PPB-X2													
13	SS-83													
14	PR-980	1												
15	SP-79-2278	0.8000	1											
16	Irv-93-1030	0.5714	0.6000	1										
17	H-57-5174	0.7428	0.8285	0.6000	1									
18	CP-51-21	0.8000	0.7714	0.5428	0.7142	1								
19	Mex-69-1460	0.6571	0.7428	0.4571	0.8000	0.7428	1							
20	H-58-4748	0.7142	0.7428	0.5142	0.6285	0.6857	0.6000	1						
21	SP-72-6163	0.6000	0.6285	0.5714	0.6857	0.6285	0.6571	0.7142	1					
22	HJ-57-41	0.8000	0.7142	0.5428	0.7714	0.7714	0.7428	0.7428	0.6285	1				
23	Irv-93-770	0.4285	0.4000	0.8000	0.4000	0.3428	0.3142	0.4285	0.5428	0.4571	1			
24	M-4	0.6571	0.6285	0.5142	0.7428	0.8000	0.8285	0.6000	0.7714	0.7428	0.4285	1		
25	N-55-805	0.6571	0.5714	0.4000	0.5714	0.6285	0.5428	0.6571	0.7714	0.5714	0.4285	0.6571	1	
26	N-55-1164	0.4285	0.4571	0.2285	0.4571	0.4571	0.5428	0.3714	0.3714	0.4000	0.2571	0.5428	0.5428	1
27	N-56-42	0.6285	0.6000	0.4285	0.6000	0.6571	0.5714	0.6857	0.8000	0.5428	0.4000	0.7428	0.9142	0.5142
28	M-442-51	0.7714	0.7428	0.5714	0.7428	0.7428	0.7714	0.7142	0.7142	0.8571	0.4857	0.7142	0.5428	0.3714
29	R-570	0.4000	0.3714	0.8571	0.4285	0.4285	0.5142	0.4000	0.4000	0.4285	0.2857	0.5142	0.5714	0.8000
30	R-579	0.4571	0.4285	0.2000	0.4285	0.4285	0.5142	0.3428	0.2857	0.4285	0.3428	0.5142	0.4571	0.9142
31	AUS-2	0.5714	0.4857	0.3714	0.6000	0.6571	0.5142	0.5714	0.7428	0.5428	0.4000	0.6857	0.9142	0.5714
32	AUS-3	0.5714	0.4857	0.3142	0.5428	0.6000	0.5142	0.5714	0.7428	0.4857	0.3428	0.6857	0.9142	0.6285
33	AUS-5	0.8571	0.8285	0.6571	0.8285	0.8285	0.7428	0.8000	0.7428	0.8857	0.5142	0.7428	0.5714	0.2857
34	AUS-8	0.6285	0.7142	0.4285	0.6571	0.6000	0.6285	0.8000	0.6857	0.6571	0.4000	0.6285	0.6285	0.4571
35	2053-BEST	0.7428	0.7142	0.5428	0.7714	0.7714	0.8000	0.6857	0.6857	0.9428	0.4000	0.8000	0.5714	0.4571
36	Q-96	0.6285	0.4857	0.3714	0.5428	0.7142	0.5714	0.7428	0.6285	0.6571	0.4000	0.6857	0.6857	0.5142
37	Q-100	0.7714	0.6857	0.5142	0.7428	0.7428	0.7714	0.7142	0.6000	0.9714	0.4285	0.7142	0.5428	0.4285
38	Q-190	0.5142	0.5428	0.3142	0.5428	0.4285	0.5714	0.5142	0.5714	0.5428	0.4000	0.5714	0.6857	0.7428

Table 3. Genetic similarity of 38 sugarcane genotypes calculated according to Nei (1978) using polymorphic RAPD markers (continued)

No.	Genotipe	N-56-42	M-442-51	R-570	R-579	AUS-2	AUS-3	AUS-5	AUS-8	2053-BEST	Q-96	Q-100	Q-190
1	PSGM-92-2290												
2	BL-666												
3	PSGM-92-2075												
4	BW-3605												
5	PHIL-71-15												
6	F-01												
7	MT-72												
8	TC-15												
9	842388												
10	GP-06												
11	BO-645												
12	R3-PPB-X2												
13	SS-83												
14	PR-980												
15	SP-79-2278												
16	Irv-93-1030												
17	H-57-5174												
18	CP-51-21												
19	Mex-69-1460												
20	H-58-4748												
21	SP-72-6163												
22	HJ-57-41												
23	Irv-93-770												
24	M-4												
25	N-55-805												
26	N-55-1164												
27	N-56-42	1											
28	M-442-51	0.5142	1										
29	R-570	0.5428	0.4000	1									
30	R-579	0.4285	0.4000	0.8857	1								
31	AUS-2	0.8857	0.5142	0.6000	0.4857	1							
32	AUS-3	0.8857	0.5142	0.6571	0.5428	0.9428	1						
33	AUS-5	0.6000	0.9142	0.3142	0.3142	0.5428	0.4857	1					
34	AUS-8	0.7142	0.5714	0.4857	0.4285	0.6000	0.6000	0.6571	1				
35	2053-BEST	0.5428	0.8571	0.4285	0.4285	0.5428	0.5428	0.8285	0.6000	1			
36	Q-96	0.7142	0.5714	0.6000	0.4857	0.7142	0.6571	0.6571	0.7142	0.6000	1		
37	Q-100	0.5142	0.8857	0.4571	0.4571	0.5142	0.4571	0.8571	0.6285	0.9142	0.6285	1	
38	Q-190	0.6571	0.5714	0.6571	0.7142	0.6571	0.7142	0.4857	0.5428	0.5428	0.4857	0.5714	1

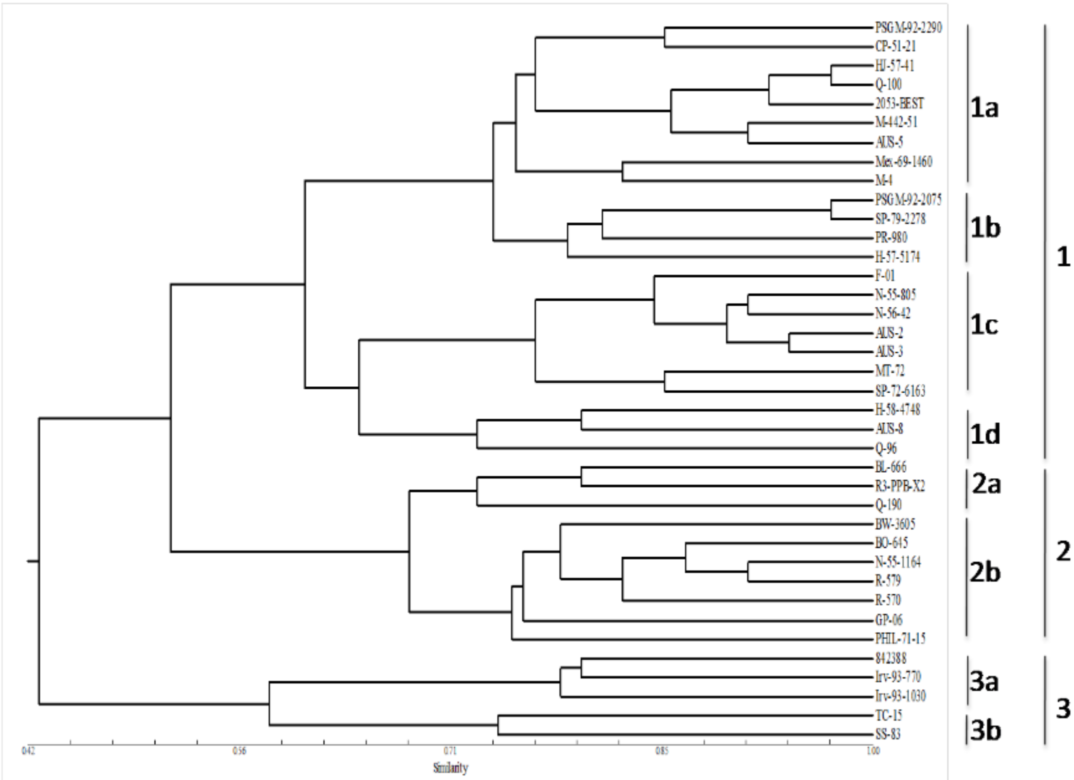

Figure 2. A dendrogram of genetic relationship of 38 sugarcane genotypes as shown by UPGMA cluster analysis based on polymorphic RAPD markers.

Clustering pattern of 38 genotypes were presented in a dendrogram shown in Figure 2. It showed that the population was clustered into three major groups, i.e. group 1, 2 and 3. There were 4 subgroups in group 1 (1a, 1b, Ic and Id), 2 subgroups in group 2 (2a and 2b) and 2 subgroups in group 3 (3a and 3b). The dendrogram showed that group 1 comprised genotypes of diverse origins i.e. Asia, Australia, Africa and America, while group 2 consisted of genotypes from Asia, Australia and Africa and group 3 consisted of only genotypes from Asia and America (Table 1 and Figure 2).

Some genotypes of same origin were clustered into in at least 3 different subgroups, meaning that they were genetically dissimilar. On the other hand, some other genotypes of different origin were clustered into the same subgroup, meaning that they were genetically similar.

Sugarcane germplasm collection in the world is mostly a result of nobilization. Nobilization was initiated by crossing *Saccharum officinarum* with *Saccharum spontaneum* in order to add its novel charaters to the high-sugar-content *Saccharum officinarum*. The progenies were then used as parents to produce commercial sugarcane clones. Therefore, the genetic base of the breeding population of sugarcane was narrow, causing slow breeding progress. This narrow genetic base was maintained by the tendency of sugarcane breeders to use parents that often produce elite progenies in the next crossings.

Sugarcane germplasm collection used in this research consisted of sugarcane commercial clones of diverse origins, namely from Australia, Africa, America, and Asia, and they all belong to *Saccharum officinarum* (Table 1). For breeding purpose, knowing the genetic similarity of the genotypes was very important to design an effective breeding program that takes advantage of heterosis. This is particularly crucial for sugarcane since commercial sugarcane has relatively narrow genetic base. Our finding showed that average genetic similarity between genotype was 57%, or the genetic distance was 43%. This figure was comparable to what reported by Khan *et al.*, (2009), Tabasum *et al.*, (2010), and Devarumath *et al.*, (2012) who reported that the genetic distance of 39%, 42% and 49%, respectively. Nair *et al.*, (2002) reported lower average pairwise genetic distance for their sugarcane germplasm collection i.e. 29%. This might be caused the less use of diverse population for their study because they used sugarcane genotypes originated from only one region, i.e India. Lower genetic distance was also reported by Saravanakumar *et al.*, (2014), Govindaraj *et al.*, (2011), and Kawar *et al.*, (2009), i.e 28%, 17%, and 13%, respectively. This might also be caused by the use of less diverse population, which consisted of sugarcane hybrids producing high biomass (Saravanakumar *et al.*, 2014), those grown in Peninsular and East coast zones of tropical India (Govindaraj *et al.*, 2011), and those originated from Coimbatore (Kawar *et al.*, 2009).

Our finding showed that UPGMA dendrogram divided the sugarcane population into 3 major groups and 8 subgroups. Each major group consisted of genotypes of different geographical origins. The dendrograms grouped genotypes from Australia in subgroup 1a, 1c, 1d, and 2a, Africa in subgroup 1a, Ic, and 2b, America in subgroup 1a, 1b, 1c, and 3a, and Asia in subgroup 1a, 1b, 1c, 2a, 2b, 3a and 3b. This indicated that even though genotypes came from the same geographical origin, they could be genetically distant. This also indicated that even though genotypes came from different geographical origin, they could be genetically similar.

While this clustering could not be explained because the parentage data for each genotype was in a shortage condition, and the dendrogram was very useful for sugarcane breeders to choose parents for hybridization. Parental selection was done not only on the basis of characters of interest but also of genetic similarity between parents. Hybridization of genetically-distant parents would most likely result in heterosis. The average genetic distance of 43% in this study demonstrated that the genetic base of the population was narrow. Therefore, parents for hybridization should be strictly selected so as to maximize heterosis. Based on the dendrogram , group 3 was actually a distinct group; group 1 and 2 cluster in one group. Based on Table 3, average genetic similarity between group 1 and 2 was 49% (genetic distance of 51%), while that between group 3 and group 1 and 2 was 44% and 41% (genetic distance of 56% and 59%), respectively. Therefore, on the basis of genetic similarity, the genotypes belonging to group 3 were good candidates for parents to be hybridisized with genotypes in group 1 and 2. In fact, the least genetically-similar genotypes were between 842388 (group 3) and BL-666 (group 2) and between Irv-93-1030 (group 3) and BL-666 (group 2), which was having genetic similarity of 17%, or genetic distance of 83%.

CONCLUSIONS AND SUGGESTIONS

The average genetic similarity among 38 sugarcane genotypes under study was 57%, which demonstrating that the genetic base of the population was narrow. Therefore, to maximize heterosis effect in a sugarcane breeding program, parents for crossing should be strictly selected on the basis of their genetic similarity in addition to their desired characters such as cane yield, sugar recovery, adaptability and resistance to pests and diseases. A dendrogram constructed using genetic similarity data showed that sugarcane genotypes clustered into 3 major groups (group 1, 2 and 3), in which group 3 was considered a distinct one. Therefore to maximize heterosis, the genotypes belonging to this group were suggested to be selected as parents to be crossed with genotypes in either group 1 and 2.

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