MOLECULAR CHARACTERIZATION AND GENETIC SIMILARITY OF THREE SUGARCANE GENOTYPES

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Sugarcane (*Saccharum* spp.) is the most important sugar-producing crop in the world (Heinz, 1987). In Egypt, sugarcane has been planted since 1850. It is cultivated in four governorates i.e. Aswan, Quena, Sohag and El-Minia. Modern sugarcane cultivars are complex polyploidy, which may contain over 100 chromosomes (Heinz, 1987; Roach and Daniels, 1987).

The Egyptian germplasm contain local genotypes and genotypes imported from different breeding stations around the world. Genotypes G.T. 54-9 and G 84-47 were developed and released in Egypt and Phil 8013 cutting was imported and evaluated under Egyptian conditions. Many investigators studied these genotypes under different environmental conditions (El-Sogheir *et al.,* 2006; Mohamed and El-Taib, 2007). Genotypes G.T. 54-9, G 84-47 and Phil 8013 differed in their genetic potential for yield and its components (stalk height, diameter and weight) as well as stalk number, Brix, sucrose%, sugar recovery% and sugar yield (ton/fed). Some genotypes significantly exceeded the check variety in some traits but were lower in other traits (El-Sogheir *et al.,* 2006; Mohamed and El-Taib, 2007).

Molecular biology provides additional technology integrated into conventional plant breeding in order to promise faster genetic gains. These new techniques are not intended to replace conventional breeding methods, but rather to facilitate and supplement crop improvement. Molecular screening procedures have yielded great benefits for many sugarcane breeding programs, with regards to disease testing by isozyme and protein analyses; and by DNA markers (Paran *et al.,* 1991; Leon *et al.,* 2001; Alvi *et al.,* 2008; Ahmed and Khaled, 2009).

The RAPD or randomly amplified polymorphic DNA (Williams *et al.*, 1990) technique, which is used in this study, allows random amplification of DNA sequences throughout the entire genome and, therefore, is very convenient for genetic diversity. RAPD markers have been successfully used to measure genetic relationships of sugarcane, *Saccharum* spp (Leon *et al*., 2001). Moreover, RAPD markers have proved useful in determining genetic relationships among sugarcane cultivars (Leon *et al.*, 2001), in determining genetic difference between resistant and susceptible sugarcane genotypes (Alvi et *al.*, 2008) and in identifying hybrids in

a "*Saccharum officinarum* × *Erianthus fulvus*'' cross (Zhang *et al.*, 2008).

The aim of the present work was to obtain molecular profiles and determine the quality traits of three sugarcane genotypes used in breeding program of the Sugar Crops Research Institute (SCRI) in order to maximize cane and sugar yields/feddan (fed = 4200 m^2).

MATERIALS AND METHODS

Filed experiment

The present study was carried out during the 2006/2007 and 2007/2008 growing seasons using three sugarcane genotypes (*Saccharum* spp.): G.T. 54-9, G 84-47 and Phil. 8013 (Table 1). The experimental plot area was 60 m^2 (12 m in width and 5 m in length). Each plot contained 12 plants with inter-row spacing of 100 cm. Dual rows of three-budded cane seeds were used in planting. A split plots design with three replications was used. Sugarcane genotypes were planted as plant cane crop and harvested at twelve months of age in both seasons. Recommended NPK fertilizers were added at rates of 210 kg N (as urea 46.5% N), 45 kg P_2O_5 (as calcium super phosphate 15.5% P_2O_5) and 48 kg K_2O (as potassium sulphate, 48% K_2O)/fed. Phosphorus fertilizer was applied during seedbed preparation. Nitrogen and potassium fertilizers were added in two equal doses after two and three months from planting. The other agricultural practices were followed as recommended by the SCRI.

Recorded data

1. Number of stalks/m^2. At harvest, a sample of twenty millable cane stalks from each plot was taken to determine the following traits:
2. Stalk height (cm) was measured from land level till point of visible dewlap.
3. Stalk diameter (cm) was determined at the middle part of the stalk..
4. Brix % in cane juice was determined using a "Brix Hydrometer" according to A.O.A.C. (1995).
5. Sucrose % in cane juice was determined using a "Saccharimeter" according to A.O.A.C. (1995).
6. Sugar recovery percentage was calculated according to the following equation as described in Yadav and Sharma (1980). Sugar recovery % = [sucrose % -0.4 (Brix % -sucrose %)] x 0.73.
7. Cane yield (ton/fed) was calculated based on plot area.
8. Sugar yield (ton/fed) was estimated as follows: Sugar yield (ton/fed) = cane yield (ton/fed) x sugar recovery %.

The collected data were subjected to proper statistical analysis of split plot design according to Bernardo (2002). Differences between means were compared using Least Significant Difference (LSD) and declared significant at $P \leq 0.05$. A combined analysis for the two seasons was done according to Bernardo (2002).

DNA isolation and RAPD-PCR analysis

DNA was isolated from 3-week old seedlings according to the method de-

scribed by Khaled and Esh (2008). Twenty-four random primers were used for RAPD- amplification (Table 2). Reaction conditions were optimized according to Sambrook *et al.* (1989). The PCR products were fractionated on agarose gel (1.2%) in TAE after staining with 0.2 mcg/ml ethidium bromide. A 100 bp ladder was used as a DNA marker to allow precise scorings of the bands.

Genetic similarity and cluster analysis

The genetic similarity between genotypes was assessed on the basis of the Dice similarity coefficient and complemented with a UPGMA-based cluster analysis according to TotalLab software package v. 2009 supplied by Nonlinear Dynamics Company. The banding patterns obtained with the 24 RAPD primers were scored and converted to binary values of (1) and (0) for the presence and absence of bands, respectively. The binary matrix was analyzed with TotalLab software to estimate genetic similarity indices among the three sugarcane genotypes. Pairwise comparisons of RAPD profiles resulted in a similarity matrix used to develop a consensus tree.

Genotype-specific markers, Genetic similarity and cluster analysis

The banding patterns obtained with the 24 RAPD primers were scored and converted to binary values of (1) and (0) for the presence and absence of bands, respectively. The binary matrix was analyzed with TotalLab software package v. 2009 supplied by Nonlinear Dynamics Company to develop a consensus tree and estimate their similarity indices for the three genotypes.

RESULTS AND DISCUSSION

Field experiment

Table (3) shows the means of the two growing season for the studied traits of the three sugarcane genotypes. Data revealed that the three sugarcane genotypes differed significantly in all studied traits. Genotype G.84/47 surpassed the others in the number of plants/m^2, followed by G.T.54/9 and Phil.8013; however, Phil 8013 surpassed the two genotypes in the sugar recovery%, G.T. 54-9 surpassed the other genotypes in stalk diameter, brix%, sucrose% cane yield and sugar yield. The previous results were in harmony with data recorded by El-Sogheir *et al.* (2006) and Mohamed and El-Taib (2007).

Genotype-specific markers based on RAPD analysis

Assessing variability and identification of available germplasm are essential components of crop improvement programs. Knowledge of the genetic distances among different genotypes is very useful for genetic improvement (Ceron and Angel, 2001). The RAPD-PCR technique has been used successfully in this regard. RAPD-PCR amplification patterns resolved varying degrees of polymorphisms between the three sugarcane genotypes considered in this study. Only seven primers produced polymorphic amplification products: OP-A01, OP-A04, OP-A07, OP-B07, OP-B10, OP-O10 and OP-O14. A

total number of 44 amplified fragments were obtained with the seven polymorphic RAPD primers for an average of 6.3 bands per primer. These polymorphic bands can be used as positive or negative molecular markers for Brix, sucrose, sugar recovery, cane yield and sugar yield (Table 4). The dendrogram of the genetic distances is shown in Fig. (2).

The seven primers showed high polymorphism in such a complex genome as that of sugarcane. That agreed with Welsh and McClelland (1990), who indicated that simple and reproducible fingerprints of complex genomes can be generated using single 10-mer primers and PCR. Twenty-nine genotype-specific markers were found, suggesting that this set of RAPD primers would be useful for genotype identification in sugarcane (Tables 4 and 5). G.T. 54-9, G 84-47, and Phi1 8013 exhibited 6, 9 and 14 genotype-specific fragments, respectively. These results confirmed the importance of using RAPD analysis for genotypic characterization, with specific markers giving informative bands that can discriminately distinguish all tested species. Similar findings were obtained by Fahmy *et al.* (2008).

Genetic similarity and cluster analysis based on RAPD markers

Genetic similarity indices among the three genotypes were 9% (G.T.54-9 and Phil 8013), 22% (G 84-47 and Phil 8013), and 37% (G.T. 54-9 and G 84-47). These results suggested a relatively wide genetic diversity among these genotypes, particularly between G.T.54-9 and Phil 8013 (currently grown commercially in Egypt).

These results disagreed with the study of Fahmy *et al.* (2008), in which the same marker system (RAPD) revealed higher genetic similarities between G.T. 54-9 and G 84-47 (66%), between G 84-47 and Phi1 8013 (69%), and between G.T.54-9 and Phi1 8013 (58%). A dendrogram, representing the relationships among the three genotypes, indicated that the genotype Phil 8013 was the most diverse among the three sugarcane genotypes (Fig. 2).

By capturing the closeness of the G.T. 54-9 and G 84-47 lines and grouping Phil 8013 (derived from a different breeding program) as an outcast, these seven primers can provide an additional discriminatory power for genetic diversity and crossing of the working germplasm in our breeding program.

SUMMARY

The molecular characterization and genetic similarity of three sugarcane *Saccharum* sp. genotypes (G.T54-9, G.84-47 and Phil 8013) were assessed by detecting polymorphisms with 24 RAPD primers. The genetic similarity between genotypes was assessed on the basis of the Dice similarity coefficient and complemented with a UPGMA-based cluster analysis. Only seven primers produced polymorphic amplification products: OP-A01, OP-A04, OP-A07, OP-B07, OP-B10, OP-O10 and OP-O14. A total number of 44 ampli-

fied fragments were obtained with the seven polymorphic RAPD primers for an average of 6.3 bands per primer. These polymorphic bands can be used as positive or negative molecular markers for °Brix, sucrose, sugar recovery, cane yield and sugar yield. The three sugarcane genotypes were divided into two clusters, the first one contains G.T. 54-9 and G. 84-47 genotypes; and the second contain Phil 8013. Genetic diversity was lowest between G.T. 54-9 and G. 84-47 (have the same mother) but highest between G.T.54-9 and Phi1 8013 (two commercially grown genotypes). The RAPD-derived genetic similarity indices ranged from 9 % between G.T.54-9 and Phi1 8013 to 37% between G.T. 54-9 and G 84-47. These results suggested a relatively wide genetic diversity among these genotypes, particularly between the two genotypes currently grown commercially (G.T.54-9 and Phi1 8013). By capturing the closeness of the G.T. 54-9 and G 84-47, and grouping Phil 8013 (derived from a different breeding program) as an outcast, these seven primers can provide an additional discriminatory power for genetic diversity and crossing of the working germplasm in our breeding program.

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Table (1): Code numbers, names, pedigrees and origins of the three sugarcane genotypes.

Code number	Variety name	Pedigree			Source of seed	Characteristics
		Female	X	Male		
1	G.T. 54-9	NCO 310	X	F 37-925	Seed fuzz from Taiwan	Good yield & high sucrose
2	G 84-47	NCO 310	X	?	Local seed fuzz	Good yield, good sucrose and early maturity
3	Phil 8013	CAC 71-312	X	Phil 642227	Seed cutting from The Philippines	Good yield & good sucrose

Table (2): Codes sequences and GC% for 24 random primers used in RAPD-PCR analysis.

Serial number	Primer code	Sequence	GC %	Serial number	Primer code	Sequence	GC %
1	OP-A01	5`-CAG GCC CTT C-3`	70	13	OP-B14	5`-TCC GCT CTG G-3`	70
2	OP-A03	5`-CAG GCC TGA C-3`	70	14	OP-B15	5`-GGA GGG TGT T-3`	60
3	OP-A04	5`-AAT CGG GCT G-3`	60	15	OP-B17	5`-TTT CCC ACG G-3`	60
4	OP-A06	5`-GGT CCC TGA C-3`	70	16	OP-B19	5`-ACC CCC GAA G-3`	70
5	OP-A07	5`-GAA ACG GGT G-3`	60	17	OP-B20	5`-GGA CCC TTA C-3`	60
6	OP-A08	5`-GTG ACG TAG G-3`	60	18	OP-C10	5`-TGT CTG GGT G-3`	60
7	OP-A09	5`-GGG TAA CGC C-3`	70	19	OP-C13	5`-AAG CTC GTC G-3`	60
8	OP-A17	5`-GAC CGC TTG T-3`	60	20	OP-D08	5`-GTG TGC CCC A-3`	70
9	OP-B07	5`-GGT GAC GCA G-3`	70	21	OP-D14	5`-CTT CCC CAA G-3`	60
10	OP-B09	5`-TGG GGG ACT C-3`	70	22	OP-O10	5`-TCA GAG CGC C-3`	70
11	OP-B10	5`-CTG CTG GGA C-3`	70	23	OP-O13	5`-GTC AGA GTC C-3`	60
12	OP-B12	5`-CCT TGA CGC A-3`	60	24	OP-O14	5`-AGC ATG GCT C-3`	60

Table (3): Means of the eight characters under investigation of the three sugarcane genotypes (combined over the two seasons).

Traits	G.T54-9	G.84-47	Phil. 8013	Mean	LSD at 5% level
Number of stalks/m2	11.51	13.49	9.94	11.65	1.43
Stalk height (cm)	278.04	274.71	266.37	273.04	7.32
Stalk diameter (cm)	2.90	2.48	2.97	2.78	1.66
Brix %	22.70	22.08	21.28	22.02	1.50
Sucrose %	16.13	15.94	15.82	15.76	0.35
Sugar recovery %	9.86	9.84	9.95	9.67	0.35
Cane yield (ton/fed)	59.25	56.13	54.91	56.76	1.45
Sugar yield (ton/fed)	6.01	5.70	5.52	5.88	0.25

Table (4): Number of amplified fragments and specific markers of the three sugarcane genotypes using RAPD analysis.

Primer	TAF	G.T. 54-9		G 84-47		Phil 8013		TSM
		AF	SM	AF	SM	AF	SM	
OP-A01	9	4	2	2	0	3	3	5
OP-A04	4	0	0	0	0	4	4	4
OP-A07	3	0	0	3	3	0	0	3
OP-B07	6	2	1	1	0	3	2	3
OP-B10	6	2	2	2	1	2	1	4
OP-O10	9	1	1	6	5	2	1	7
OP-O14	7	2	0	2	0	3	3	3
Total	44	11	6	16	9	17	14	29

Table (5): Molecular characterization of the three sugarcane genotypes based on the specific markers of RAPD analysis.

Genotypes	RAPD primer	Band size (bp)	Genotypes	RAPD primer	Band size (bp)
G.T. 54-9	OP-A01	3150, 559	Phil 8013	OP-A01	755, 282, 181
	OP-B07	310		OP-A04	689, 733, 767, 949
	OP-B10	375, 273		OP-B07	560, 336, 205
	OP-O10	527		OP-B10	1058, 267, 223
G 84-47	OP-A07	786, 710, 602		OP-O10	922
	OP-B10	847, 531, 223, 136		OP-O14	1045, 790, 214
	OP-O10	859, 794, 746, 721, 676			

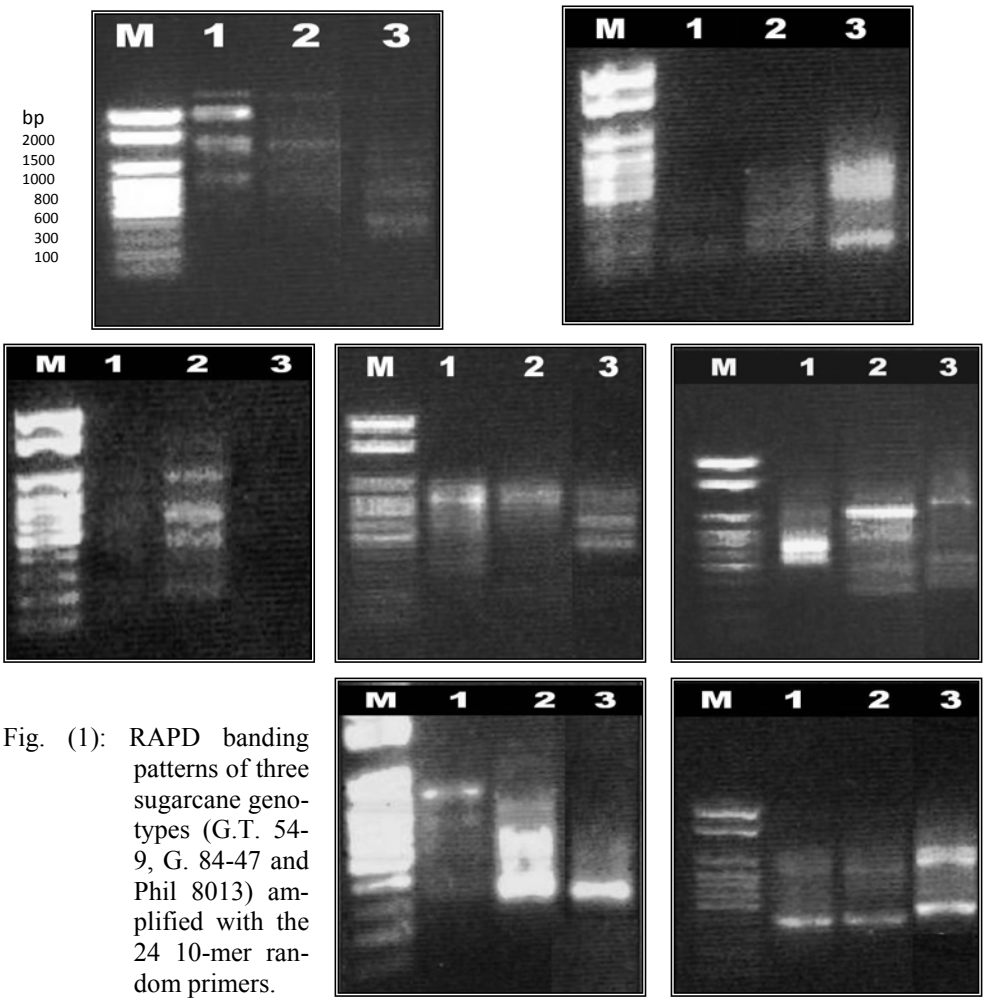

Fig. (1): RAPD banding patterns of three sugarcane genotypes (G.T. 54-9, G. 84-47 and Phil 8013) amplified with the 24 10-mer random primers.

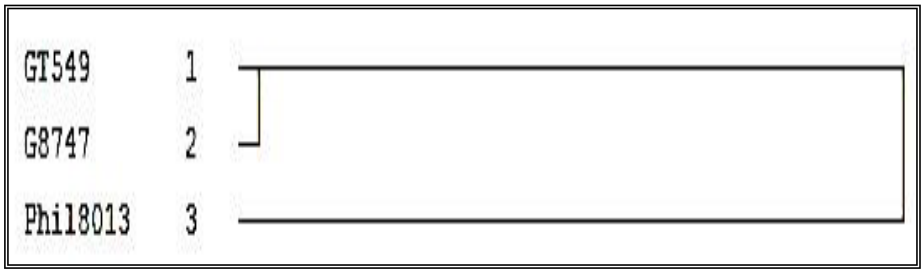

Fig. (2): Dendrogram illustrating the relationships among the three sugarcane genotypes based on similarity indices derived from RAPD analysis.