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Molecular Profiling and Genetic Diversity in Sugarcane using RAPD

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ABSTRACT

The genetic diversity of six sugarcane *Saccharum* sp. genotypes (G.T54-9, G.84-47, G.98-28, G.95-21, G.95-19 and Phil8013) was 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 three primers produced polymorphic amplification products: OP-A01, OP-A07 and OP-B07. A total number of 130 amplified fragments were obtained with the three polymorphic RAPD primers for an average of 43 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 six sugarcane genotypes were divided into four clusters. Genetic diversity was lowest between G.T. 95-21 and G 95-19 (two sister lines) but highest between G.T.54-9 and Phi1 80-13 (two commercially grown genotypes). The RAPD-derived genetic similarity indices ranged from 10% between G.T.54-9 and Phi1 8013 to 87% between G.T. 95-21 and G 95-19. By capturing the closeness of the two sister lines and grouping Phil 8013 (derived from a different breeding program) as an outcast, these three primers can provide an additional discriminatory power for genetic diversity and crossing of the working germplasm in our breeding program.

Keywords: genetic similarity, polymorphism, RAPD, UPGMA

INTRODUCTION

Biotechnology is considered as a serious tool to increase agricultural productivity in the context of sustainable agriculture (Tecson 2002). Integration of molecular biology as an additional technology into plant breeding promises faster genetic gains than are possible with conventional approaches. 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; Zambrano *et al.* 2003; Alvi *et al.* 2008; Ahmed and Khaled 2009). More specifically, molecular markers such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) have recently shown excellent potential in assisting in the identification of quantitative trait loci (QTLs) (Stuber 1992; Ming *et al.* 2002; Fratini *et al.* 2007; Khaled *et al.* 2007).

The RAPD or random amplification of polymorphic DNA (Williams *et al.* 1990) technique, which is used in this study, allows random amplification of DNA sequences throughout the entire genome and, for so, is very convenient for genetic diversity. RAPD polymorphisms result from nucleotide base changes, i.e., from insertions or deletions that alter the primer-binding sites (Williams *et al.* 1993). These products of amplification can be polymorphic and are used as genetic markers (Tingey and Del Tufo 1993). RAPD markers have been successfully used to measure genetic relationships of cultivated species such as wheat (*Triticum aestivum* L.), sugarcane (*Saccharum* spp.), and cotton (*Gossypium arboreum*) (Cao *et al.* 2000; Leon *et al.* 2001; Kumar *et al.* 2008). PCR amplified DNA has been used to identify varieties (Piperidis *et al.* 2004) and understanding the genealogy of sugarcane varieties in breeding programmes (Garcia *et al.* 1997). 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 assess the discriminatory power of a set of RAPD primers in determining genetic diversity among six sugarcane genotypes, of which three are commercial cultivars and the other three are advanced selections in the Egypt breeding program.

MATERIALS AND METHODS

Experiment to determine genetic diversity of sugarcane

The present study was carried out at the farm, greenhouse, and laboratories of the Department of Breeding and Genetics, Sugar Crops Research Institute, Agriculture Research Center, Giza, and Plant Genetic Resource Department (PGRD, Arid Land Cultivation and Development Research Institute (ALDRI), Mubarak City for Scientific Research and Technology Applications. Six sugarcane genotypes (G.T54-9, G.84-47, G.98-28, G.95-21, G.95-19 and Phil8013) were included in this study. The selected genotypes, along with their parentages, are presented in **Table 1**.

DNA isolation and RAPD-PCR analysis

DNA was isolated from 3-week old seedlings according to the method described by Khaled and Esh (2008). Twenty-four random primers were used for RAPD- amplification (**Table 2**).

Reaction conditions were optimized according to Maniatis *et*

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Research Note

Table 1 Code numbers, names, pedigrees and origins of the six sugarcane genotypes.

Variety name	Pedigree			Source of seed
	Female		Male	
Phil 8013	CAC 71-312	X	Phil 642227	Seed cutting from The Philippines
G 98-28	C 34-33	X	?	Local seed fuzz
G 95-21	Sp 79-2278	X	Sp 80-1043	Local seed fuzz
G 95-19	Sp 79-2278	X	Sp 80-1043	Local seed fuzz
G.T. 54-9	NCO 310	X	F 37-925	Seed fuzz from Taiwan
G 84-47	NCO 310	X	?	Local seed fuzz

? Unknown parent

Table 2 Name, sequences and GC % for 24 random primers used in RAPD-PCR analysis.

Primer code	Sequence (5′-3′)	GC %	Primer code	Sequence (5′-3′)	GC %
OP-A01	CAGGCCCTTC	70	OP-B14	TCCGCTCTGG	70
OP-A03	CAGGCCTGAC	70	OP-B15	GGAGGGTGTT	60
OP-A04	AATCGGGCTG	60	OP-B17	TTTCCCACGG	60
OP-A06	GGTCCCTGAC	70	OP-B19	ACCCCCGAAG	70
OP-A07	GAAACGGGTG	60	OP-B20	GGACCCTTAC	60
OP-A08	GTGACGTAGG	60	OP-C10	TGTCTGGGTG	60
OP-A09	GGGTAACGCC	70	OP-C13	AAGCTCGTCG	60
OP-A17	GACCGCTTGT	60	OP-D08	GTGTGCCCCA	70
OP-B07	GGTGACGCAG	70	OP-D14	CTTCCCCAAG	60
OP-B09	TGGGGGACTC	70	OP-O10	TCAGAG CGCC	70
OP-B10	CTGCTGGGAC	70	OP-O13	GTCAGAGTCC	60
OP-B12	CCTTGACGCA	60	OP-O14	AGCATGGCTC	60

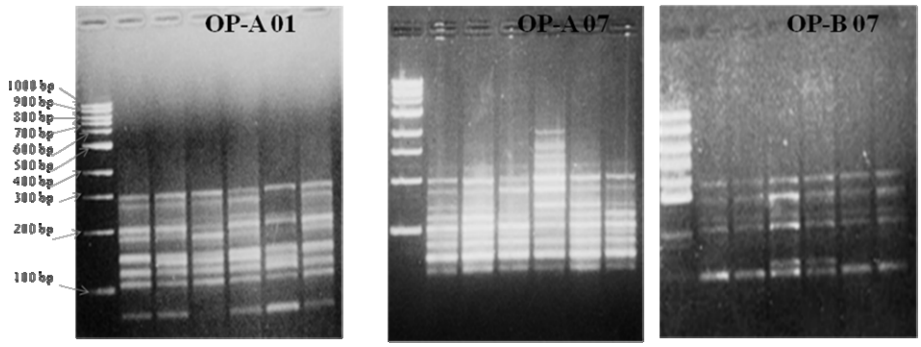

Fig. 1 RAPD banding patterns of six sugarcane genotypes (GT 54-9, G 95-21, G 95-19, G 98-28, G 84-47 and Phil 8013) amplified with 10-mer random primers.

al. (1982) and 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 Co. 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 to estimate genetic similarity indices among the six sugarcane genotypes. Pairwise comparisons of RAPD profiles resulted in a similarity matrix used to develop a consensus tree.

RESULTS AND DISCUSSION

Genotype diversity based on RAPD analysis

Assessing variability and identification of available germplasm is an essential component of crop improvement programs. Knowledge of the genetic distances among different varieties is very useful in planning useful crosses in order to obtain greater genetic gains (Ceron and Angel 2001). RAPD-PCR was used successfully in this regard.

The RAPD-PCR amplification patterns observed in this study resolved varying degrees of polymorphisms between the six sugarcane genotypes (**Fig. 1**). A total number of 130 amplified fragments were obtained with the three polymorphic RAPD primers (**Table 3**), with an average of 43 bands per primer. A total number of 51 DNA fragments were amplified with primer OP-A01 in the six genotypes, with molecular weights ranging from 33 to 190 base pairs (bp). A total of 51 DNA fragments were also scored with primer OP-A07 with molecular weights ranging from 23 to 292 bp. Primer OP-B07 amplified 28 DNA fragments with molecular weights ranging from 115 to 414 bp (**Fig. 1**). The RAPD-derived genetic similarity indices ranged from 10 to 87%. The highest similarity value (87%) was recorded between G.T. 95-21 and G 95-19, whereas the lowest value (10%) was found between G.T.54-9 and Phi1 8013.

Genetic similarity indices among the six genotypes were 10% (G.T.54-9 and Phi1 8013), 24% (G 84-47 and Phi1 8013), 39% (G.T. 54-9 and G 84-47) and 87% (G 95-

Table 3 Total number of amplified fragments, polymorphic fragments and percentage polymorphism in the six sugarcane genotypes using RAPD analysis.

Primer name	TAF[a]	PF[b]	P %[c]
OP-A01	51	39	76.5
OP-A07	51	44	86.3
OP-B07	28	28	100
Total	130	111	85.4

[a] Total amplified fragments
[b] Polymorphic fragments
[c] Percentage polymorphism

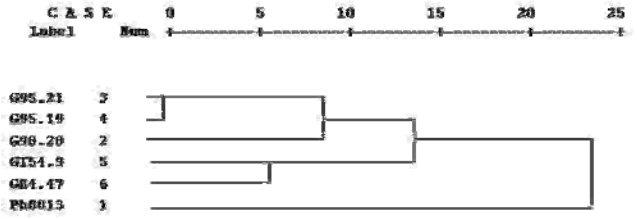

Fig. 2 Dendrogram representing the relationships among six sugarcane genotypes based on similarity indices derived from RAPD analysis.

21 and G 95-19). These results suggested a relatively wide genetic diversity among these genotypes, particularly between those (G.T.54-9 and Phi1 8013) currently grown commercially. These results were in agreement with those obtained by Ahmed and Khaled (2008, 2009), who demonstrated that 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. In contrast, these results disagreed with the study of Eman Fahmy *et al.* (2008), in which the same primers 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%).

Cluster analysis based on RAPD profiling

A dendrogram, representing the relationships among the six genotypes, indicated that Phil 8013 was the most diverse among the studied sugarcane genotypes (**Fig. 2**). The dendrogram separated the six sugarcane genotypes into two main clusters, where genotype Phil 8013 was placed in a separate cluster, while the remaining genotypes constituted the second cluster. The second cluster was further subdivided into two sub-clusters. The first sub-cluster was subdivided into two sub-sub-clusters; one of them included genotypes G 95-21 and G 95-19, while the other contained genotype G 98-28 only. Genotypes G.T 54-9 and G 84-47 occurred in the second sub-cluster. These results were in agreement with the pedigree information of these genotypes (**Table 1**), emphasizing the advantages of using RAPD system in order to obtain high resolution profiles that discriminate among closely related and different genotypes.

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