Article

Detection of Genetic Similarity of Sugarcane Genotypes

by

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Abstract

The present work was conducted at the El-Mattana Agricultural Research Station, Quena Governorate during the 2006/2007 and 2007/2008 seasons to assess the effect of row spacing (80, 100 and 120 cm) on three sugarcane (Saccharum spp.) genotypes (G.T.54-9, G.84-47 and Phil 8013) and to detect the genetic similarity among these genotypes. A split plots design with four replications was used. The results indicated that planting sugarcane in rows spaced at 80-cm apart attained significant increases in stalk height, number of millable stalks, cane and sugar yields/fed* when compared with 100 and 120 cm spacings. The 120-cm row spacing produced the largest barrels Total soluble solids, sucrose and sugar recovery % was not affected by row spacing. The tested sugarcane genotypes differed significantly in all traits studied, except in height, diameter of stalks, and total soluble solids % in the 2nd season. G.T.54-9 yielded the highest values of stalk height, cane and sugar yields/fed, while Phil.8013 had the thickest stalks, the highest sucrose and sugar recovery percentages. The number of millable stalks was higher for G.84-47 genotype. Under the conditions of this study, planting GT.54-9 in rows spaced 80 cm apart is recommended to obtain the highest cane and sugar yields/fed. 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. G 84-47 and Phi1 8013 share 22% of their genomes. 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).

Key words:

genotypes, sugarcane ,spacing, similarity

Introduction

Sugarcane (Saccharum spp) is the most important sugar-producing crop in the world (Heinz, 1987). In Egypt, sugarcane planted since 1850. It is cultivated in four governorates i.e. Aswan, Qena, Sohag and EL-Minia. Modern sugarcane cultivars are complex polyploids, which may contain over 100 chromosomes Heinz, (1987) and Roach and Daniels, (1987). From an agronomic standpoint, many factors, such as row spacing and varieties, affect sugar cane yield. Row spacing has a direct effect on plant population, cane diameter and length, which ultimately contribute to yield. It also

plays a distinct role in the amount of solar radiation intercepted by the crop canopy, which in turn affects photosynthesis and ultimately the dry matter produced by the plant. Recently, the Sugar Crops Research Institute Giza, Egypt developed many promising varieties of sugarcane, among them G84-47 and Phil 8013. Shah-Nawaz, et al. (2000) testing sugarcane in rows of 75, 90, and 120 cm apart, found that the 75-cm spacing produced the highest number of millable stalks, whereas cane yield was maximized with the 90-cm spacing. Sucrose content in cane juice was not significantly affected by spacing. Ahmed et al. (2002) indicated that the examined inter-row spacing significantly affected number of plants/m2, cane and sugar yield with significant differences among the tested varieties. El-Geddawy, et al. (2002) found that a narrow row spacing (100 cm) produced higher number of millable canes, cane and sugar yields compared with 120 and 140-cm row spacing. Sugarcane F.153 variety produced the highest number of millable cane and cane yield compared with the other varieties. G.T.54-9 cultivar significantly surpassed the others in terms of stalk height, stalk diameter, sugar recovery %, and sugar yield. Raskar and Bhoi (2003) showed that number of millable cane were significantly higher with a 90-cm intra-row spacing compared with 30 or 60-cm. Sundara (2003) compared sugarcane cultivars Co 91010, Co 94005 and Co 94008 in rows spaced at 90, 120 or 150 cm. He found that Co 91010 recorded the highest number of stalks at harvest, commercial cane sugar % and cane yield compared with the other varieties. A spacing of 90 cm resulted in the highest number of stalks and juice quality traits were not significantly affected. Rizk et al (2004) found that sucrose was not significantly affected by the studied row distances (100, 120, and 140 cm), but thickest stalks were produced with the widest row distance. El-Shafai, and Ismail (2006) indicated that planting sugarcane in rows spaced at 80-cm apart attained significant increases in cane stalk height, number of millable , cane and sugar yields/fed compared with 100 and 120 cm. The largest barrels were recorded under the 120-cm row spacing. Sucrose and sugar recovery percentages were not significantly affected by row spacing.

Biotechnology has been used as a 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. 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 DNA markers. Molecular markers offer specific advantages in assessment of genetic diversity and in trait-specific crop improvement. Use of molecular markers in the applied breeding programs can facilitate the appropriate choice of parents to make crosses. Molecular markers have been used for studying genetic diversity, cultivar identification, and for marker-assisted selection (MAS) of major crops such as rice (Oryza sativa), maize (Zea mays), wheat (Triticum aestivum) and sugarcane. Moreover, molecular markers such as RFLP, RAPD, ISSR and SSR have recently shown excellent potential in assisting selection of quantitative trait loci (QTLs) Stuber, (1992). The RAPD marker approach Williams et al., (1990) has allowed simple, easy and less time-consuming genome analysis at DNA level when compared with RFLP. The RAPD technique, as a simple and rapid procedure, has gained worldwide acceptance Michelmore et al., (1991); Paran et al., (1991).

The aim of the present work was to determine the optimum row spacing to grow the newly developed sugarcane varieties in order to maximize cane and sugar yields/fed. The fingerprinting profiles obtained with RAPD were made to estimate the genetic similarity among the studied genotypes.

Materials and Methods

Row spacing experiment:

The present study was carried out at the El-Mattana Agricultural Research Station, Qena Governorate (Upper Egypt) in the 2006/2007 and 2007/2008 growing seasons This study included nine treatments representing the combination of

three sugarcane genotypes (Saccharum spp.) G.T54-9, G.84-47 and Phil.8013, (Table I). and three row spacing [80, 100 and 120 cm]. The experimental plot area was 60 m2 (12 m in width and 5 m in length). Each plot contained 15, 12 and 10 ridges for the inter-row spacing of 80, 100 and 120 cm, respectively. Dual rows of three-budded cane seeds were used in planting. A split plots design with four replications was used. Row spacing was allocated to the main plots while the examined varieties were distributed in the subplots. Sugarcane varieties were planted as plant cane crop in the 2nd week of March 2006 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 P2O5 (as calcium super phosphate 15.5 % P2O5) and 48 kg K2O (as potassium sulphate, 48 % K2O)/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 Sugar Crops Research Institute.

Recorded data:

1. Number of millable canes/m2.

At harvest, a sample of twenty millable cane stalks from each subplot was taken to determine the following traits:

2. Millable cane height (cm) was measured from land level till point of visible dewlap.

3. Millable cane 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 statistically analyzed according to the method of Snedecor and Cochran (1981).

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

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

DNA isolation

DNA was isolated from 3-week old seedlings according to the method described by Khaled and Esh (2008)

RAPD-PCR analysis

Reaction conditions were optimized according to Maniatis et al. (1982) and Sambrook and Maniatis (1989). The product was fractionated on agarose gel (1.2 %) in TAE buffer and was stained with 0.2g/ml ethidium bromide and a 100bp ladder was used as a DNA marker. Twenty-four random primers were used for RAPD amplification, their names and sequences as presented in Table 2.

Table 2: Name, 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

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 the SPSS software (Company, place) to develop a consensus tree and estimate their similarity indices for the three genotypes.

RESULTS AND DISCUSSION

I. Row spacing experiment:

1. Number of plants/m2

Table 3 shows that increasing row spacing from 80 up to 120 cm resulted in a significant decrease in the number of millable stalks/m2 from 13.258 to 8.696 in the plant cane and from 14.194 to 9.411 in the first ratoon crops. These results may be due to a requirement of high rate of seed setts in planting the narrow row spacings. Similar results were obtained by Shah-Nawaz, et al. (2000), Raskar and Bhoi (2003) and Sundara (2003).

Data shows that the three sugarcane genotypes differed significantly in number of millable stalks/m2. Genotype G.84/47 surpassed the others in the number of plants/m2, followed by G.T.54/9 and Phil.8013 in both the plant cane and first-ratoon crops. Genotypic differences for this trait were also detected by Gowda, et al. (2001) and Sundara (2003)

Number of millable stalks/m2 was significantly influenced by the interaction between row spacing and genotypes in the 1st season only. The genotype G.84/47 significantly surpassed the other two genotypes in all row spacings, except in the 120-cm spacing where it equals G.T.54-9.

Table 3: Number of millable stalks/m2 of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	13.427	9.623	9.523	10.858	13.89	10.617	9.85	11.452
G.84-47	14.880	11.640	9.907	12.142	16.513	12.86	10.0	13.124
Phil. 8013	11.467	9.55	6.657	9.224	12.180	10.243	8.383	10.269
Mean	13.258	10.241	8.696	10.741	14.194	11.240	9.411	11.615
LSD at 5% level for:								
Row spacing				0.438				0.912
Genotypes				1.360				1.503
Row spacing x Genotypes				0.758				N. S

2. Millable cane height

Table 4 indicates that millable cane height significantly decreased when row spacing increased from 80 to 120 cm in the plant cane only. This result may be attributed to competition among cane plants where the proportion of invisible solar radiation is so much increased than the visible solar radiation due to dense sowing (Chang, 1974). Increasing plant density usually results in an increase of stalk elongation due to competition for light. Similar results were reported by El-Geddawy, et al. (2002) and El-Shafai, and Ismail (2006).

Millable cane height was significantly affected by the genotypes effect in the plant cane only, with no significant difference, however, between G.T.54/9 and G.84/47. This result is in accordance with those reported by. El-Geddawy et al. (2002) and Ahmed et al. (2002).

The interaction effect between sugar cane genotypes and row spacing was not significant for this trait

Table 4: Millable cane height of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	300.33	293	283	292.11	309.33	287.33	283	293.22
G.84-47	302.33	289.33	281.67	291.11	288	292	281.67	287.22
Phil. 8013	284.67	279	273.67	279.11	286.67	279.33	279	281.67
Mean	295.78	287.11	279.44	287.44	294.67	286.22	281.22	287.37

LSD at 5% level for:		
Row spacing	5.254	N. S
Genotypes	7.327	N. S
Row spacing x Genotypes	N. S	N. S

3. Millable cane diameter

Data tabulated in Table 5 revealed that millable cane diameter was significantly affected by row spacing. Increasing spacing between rows from 80 to 100cm and to 120 cm increased diameter by 8.04 and 12.29% in the plant cane, and by 4.36 and 13.71% in the first ratoon crop. This result may be due to lower competition among cane plants grown in the 120-cm spacings, and consequently to better growth conditions, as compared with those grown in rows spaced 80 or 100 cm apart. These results were in harmony with those obtained by El-Geddawy, et al. (2002) and, Rizk et al. (2004)

The examined sugar cane genotypes differed in their thickness. Sugar cane genotype Phil.8013 gave the thickest diameter followed by G T.54/9 genotype. This finding was true in the plant cane and its ratoon. Otherwise, difference between the examined genotypes was significant in the plant cane only. The superiority of Phil.8013 genotype in stalk diameter may be due the low number of millable cane/m2 which in turn reflected on stalk diameter (Table 3). Similar results were reported by El-Geddawy, et al. (2002) and Ahmed et al. (2002).

Millable cane diameter was insignificantly affected by the interaction among the two studied factors in the 1st and 2nd seasons.

Table 5: Millable cane diameter of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	2.73	2.857	2.970	2.852	2.523	2.647	2.9	2.69
G.84-47	2.403	2.673	2.797	2.624	2.39	2.427	2.623	2.48
Phil. 8013	2.773	3.013	3.113	2.967	2.59	2.757	3.01	2.786

Mean	2.636	2.848	2.960	2.814	2.501	2.61	2.844	2.652

LSD at 5% level for:		
Row spacing	0.111	0.132
Genotypes	0.166	N. S
Row spacing x Genotypes	N. S	N. S

4- Brix percentage

Neither row spacing or the GxR interaction affected Brix % values in the plant and first ratoon crops.

Table 6 indicates that the evaluated sugarcane genotypes differed significantly in juice Brix% in the plant-cane crop only. Phil 8013 attained the highest values of Brix (16.11%) followed by G.T.54-9 (15.0%), and then by G. 84-47 (14.3%) Since the effects of row spacing or of the interaction were inexistent, these differences in Brix% between the studied genotypes may be mainly attributed to their genetic make-up, reflecting their specific adaptation to these conditions. These results were in agreement with those mentioned by Ahmed et al. (2002) and El-Shafai, and Ismail (2006)

Table 6: Brix percentage of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	15.12	14.83	15.077	15.009	21.887	22.107	21.967	21.987
G.84-47	14.457	14.237	14.233	14.309	21.88	20.807	20.947	21.211
Phil. 8013	15.643	16.277	16.42	16.113	23.047	23.320	23.713	23.36
Mean	15.073	15.114	15.243	15.144	22.271	22.078	22.209	22.186

LSD at 5% level for:		
Row spacing	N.S	N.S
Genotypes	1.528	N.S
Row spacing x Genotypes	N.S	N.S

5- Sucrose percentage

As for Brix %, Table 7 showed that sucrose percentage was not significantly affected by row spacing or by the interaction in either crop season. Similar results were obtained by Shah-Nawaz, et al. (2000), Sundara (2003), and by Rizk (2004) .

Figures in Table 6 showed that Phil 8013 genotype significantly surpassed the other two genotypes in sucrose percentage in the plant-cane and first-ratoon crops, with the lowest values recorded for G.84-47. Differences in this trait among genotypes may be due to growth characters as well as total soluble solids percentage of Ph.8013 genotype as mentioned before. This result was in line with those obtained by Ahmed et al. (2002) and El-Shafai, and Ismail (2006)

Table 7: Sucrose percentage of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	17.87	18.000	18.133	18.001	18.643	18.587	18.383	18.538
G.84-47	17.043	17.090	17.150	17.094	17.720	17.697	17.590	17.669
Phil. 8013	19.087	18.813	18.623	18.841	19.877	19.263	19.197	19.446
Mean	18.00	17.968	17.969	17.979	18.747	18.516	18.390	18.551

LSD at 5% level for:

Row spacing	N.S	N.S
Genotypes	0.240	0.468
Row spacing x Genotypes	N.S	N.S

6-Sugar recovery percentage

Sugar recovery percentage was not affected by row spacing or by the interaction among the two studied factors (Table 8). This result is in agreement with those reported by Sundara (2003) and El-Shafai, and Ismail (2006).

The examined genotypes differed significantly in sugar recovery percentage for the two years considered. Phil 8013 surpassed the other two genotypes with no significant difference found, however, in this trait between Phil 8013 and G.T. 54-9 in the first-ratoon crop. The superiority of Ph. 8013 genotype in this trait could be attributed to higher sucrose % recorded for that genotype (Table 7). This result is in agreement with that reported by Sundara (2003), and El-Geddawy, et al. (2002)

Table 8: Sugar recovery percentage of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	12.213	12.180	12.470	12.288	12.660	12.537	12.370	12.522
G.84-47	11.437	11.710	11.677	11.608	11.720	12.007	11.860	11.862
Phil. 8013	12.940	12.567	12.297	12.601	13.407	12.900	12.700	13.002
Mean	12.197	12.152	12.148	12.166	12.596	12.862	13.002	12.462

LSD at 5% level for:

Row spacing	N.S	N.S
Genotypes	0.188	0.522
Row spacing x Genotypes	N.S	N.S

7.Cane yield

Yield (cane and sugar) of sugar cane plants is considered the economical trait of the plants, and its final expression is the result of interactions between the genetic makeup of the cultivated variety, cultural practices, and environmental factors. Table 9 revealed that the row spacing effect, cane yield significantly decreased with an increase row width, with wider rows producing the lowest cane yield (Table 9). This can be explained by the fact that narrower rows (80 cm) in this study produced more millable stalks/m2 and the tallest cane plants (Tables 3 and 4). Planting sugarcane in 80-cm rows produced 1.89 and 8.55 % more cane yield in the plant cane and 4.01 and 6.95 % more tonnage in the ratoon crop than in the 100 or 120-cm row spacings, respectively. These results are in agreement with those reported by Shah-Nawaz, et al. (2000), Gowda, et al. (2001), El-Geddawy, et al. (2002) and El-Shafai, and Ismail (2006).

With regards to the examined genotypes differed significantly in cane yield in both the plant cane and first ratoon crops. G.T.54/9 outyielded G.84/47, and Phil.8013 by 2.309 and 1.656 ton/fed, respectively, in the plant cane, and by 3.108 and 4.056 ton/fed, respectively, in the first ratoon. Differences in cane yield among genotypes were also found by Gowda, et al. (2001) , Sundara (2003) , and El-Geddawy, et al. (2002)

Cane yield was significantly influenced by the interaction between genotypes and row spacing in both seasons. The differences between G.T.54/9 and G.84/47 varieties were significant when compared at all examined row width. Differences between GT.54-9 and Phil 8013 were not significant when compared in the first ratoon at the 80-cm row. In general, GT.54-9 planted at 80-cm or at 100-cm apart produced the highest cane yield.

Table 9: Cane yield (ton/fed) of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	52.250	52.467	48.953	51.223	54.450	54.233	51.792	53.557
G.84-47	50.933	50.233	45.576	48.914	52.220	51.600	47.528	50.449
Phil. 8013	51.917	49.467	47.316	49.567	51.000	49.400	48.104	49.501
Mean	51.700	50.722	47.282	49.901	52.557	50.449	49.141	51.147

LSD at 5% level for:		
Row spacing	0.631	0.715
Genotypes	1.504	1.412
Row spacing x Genotypes	1.093	1.238

8. Sugar yield

Results in Table 10 shows significant difference in cane yield/fed due to the row spacing. Raising the row spacing from 80 to 100 and from 80 to 120 cm. led to a decrease in sugar yield/fed of 0.078 and 0.518 as well as 0.162 and 0.582 ton/fed, in the, 1st and 2nd seasons, respectively. This result may be due to higher cane yield/fed at 80 cm spacing (Table 9), which is considered the main component of sugar yield. This is in harmony with those obtained by El-Geddawy, et al. (2002) and El-Shafai, and Ismail (2006) who reported that the difference in sugar yield between 80 and 100 cm row spacing was insignificant in both seasons. Therefore, planting sugarcane in rows spaced at 100 cm may save some canes used as seeds for planting.

The results showed a significant differences among the tested sugarcane genotypes for sugar yield. Sugarcane genotype G.T.54-9 produced the highest sugar yield/fed than the other genotypes with no statistical difference with that produced by Phil.8013. G.84-47 had the lowest sugar yield consistently in both crops. This result was in accordance with that reported by Gowda, et al. (2001), and El-Geddawy, et al. (2002).

Sugar yield was significantly affected by the interaction between the tested genotypes and row spacing (Table 10) in the plant-cane crop only. Phil 8013 produced more sugar/ton with the 80-cm row spacing and less with every 20-cm increase in width, sugar yield of G.T. 54-9 decreased only at the 120-cm spacing, causing an interaction.

Table 10: Sugar yield (ton/fed) of three sugarcane genotypes as affected by inter-row spacing

Genotypes	2006/2007 season				2007/2008 season			
	Row spacing				Row spacing			
	80 cm	100 cm	120 cm	Mean	80 cm	100 cm	120 cm	Mean
G.T54-9	6.272	6.390	6.103	6.255	6.895	6.798	6.406	6.700
G.84-47	5.827	5.917	5.322	5.688	6.119	6.195	5.632	5.982
Phil. 8013	6.656	6.216	5.778	6.217	6.838	6.371	6.066	6.425
Mean	6.252	6.174	5.734	6.053	6.617	6.455	6.035	6.369

LSD at 5% level for:		
Row spacing	0.188	0.230
Genotypes	0.172	0.331
Row spacing x Genotypes	0.326	N.S

II. 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 varieties is very useful for genetic improvement Ceron and Angel, (2001). The RAPD-PCR technique has been used successfully in this regard (Reference). RAPD-PCR amplification patterns resolved varying degrees of polymorphisms between the three sugarcane genotypes considered in this study. A total of 44 fragments were resolved across genotypes and markers (Table 11). The dendrogram of the genetic distances is shown in Fig. 2.

Table 11: 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

TAF: total amplified fragments AF: amplified fragments SM: specific markers TSM: total specific markers

A total number of 44 amplified fragments were obtained with the 24 RAPD primers. All 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 11 and 12). G.T. 54-9, G 84-47, and Phi1 8013 exhibited 6, 9 and 14 genotype-specific fragments, respectively.

Table12 : Molecular characterization of the three sugarcane genotypes based on the specific markers of RAPD analysis

Genotypes	RAPD primer	Molecular 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			

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 Fahmy, et al. (2008).

III. Genetic similarity and cluster analysis based on RAPD markers

Genetic similarity indices among the three genotypes were 9 % (G.T.54-9 and Phi1 8013), 22% (G 84-47 and Phi1 8013), and 37% (G.T. 54-9 and G 84-47). 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 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 (Fig. 2).

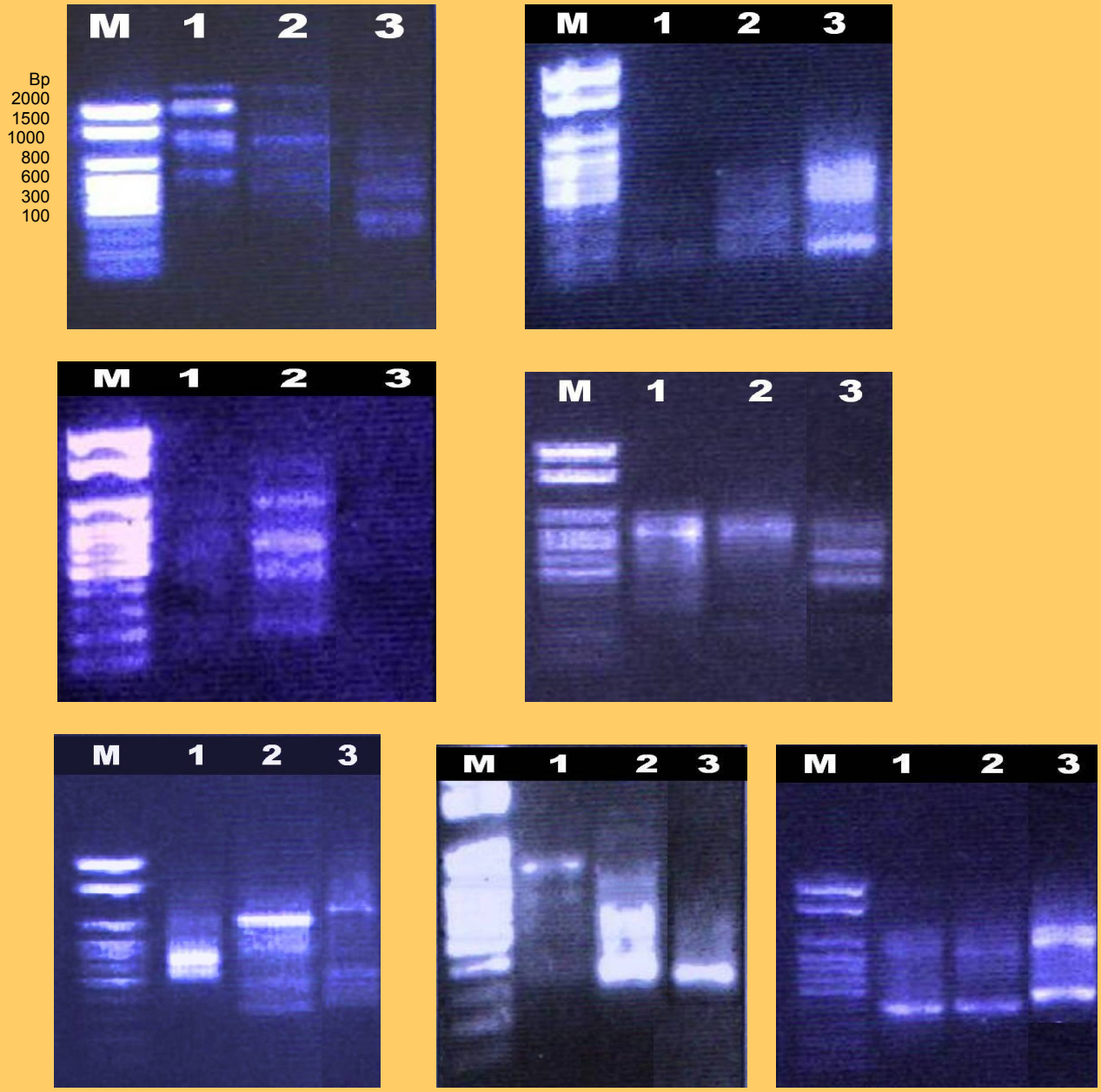

Figure (1): RAPD banding patterns of three sugarcane genotypes (GT, G, and Phil etc) amplified with the 24 10-mer random primers.

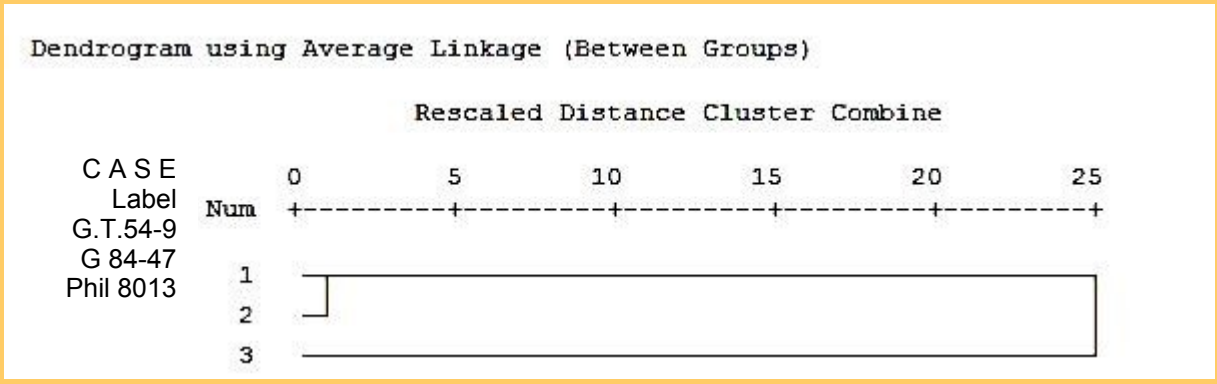

Figure (2): Dendrogram representing the relationships among three sugarcane genotypes based on similarity indices

derived from RAPD analysis

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