EVALUATION OF SIX SUGARCANE GENOTYPES UNDER THE EGYPTIAN CONDITIONS USING INTER SIMPLE SEQUENCE REPEAT (ISSR) TECHNIQUE

EL-GEDDAWY, DALIA I. H.[a*]; SAHAR A. M. SHAMSELDIN[**]; M. H. M. EBID [*]AND ELSHEIKH, A. A[*]

[*] *Sugar Crops Res. Inst., Agric. Res. Center, Giza, Egypt*

[a] *Corresponding author: Email:elgeddawydaliascientific@gmail.com:01203815776*

[**] *Botany Dept. Women's College for Arts, Sci. and Edu., Ain Shams Univ. Egypt*

Key words: ISSR, genotypes sugar cane and varieties.

The development of sugar industries depends mainly on variety improvement in most of sugarcane-producing countries. The targets of sugarcane breeding programs include the improvement of quality and quantity traits i.e., sugar content, cane yield, ratooning ability, disease resistance and maintaining satisfactory fiber levels for milling. From the economical point of view, when sugar content in cane increases, it is reflected on the increment of sugar produced from farms and mills with very little increase in marginal costs through harvesting, cane transport or milling (Jackson, 2005). Deren (1995) mentioned that most of the sugarcane varieties cultivated in the world today can be traced back to only a few common ancestors. Moreover, Edme´ *et al.,* (2005) suggested that this is may be because of the genetic bottleneck effect, where the rate of genetic gains through sugarcane crossing has been slow.

The national regional tests and integrated demonstrations of sugarcane varieties can provide quantitative data on certain characters, which are valuable to the breeders. Besides, when breeders select crossing parents from the local germplasm collection, it would be helpful to know the genetic relationship among clones of the germplasm collection and predict the promotion potential of new varieties (You *et al*., 2016). According to Hont *et al.,* (1995) and Parida *et al*., (2009), the molecular markers play an important role in uncovering the complex genetics of sugarcane and to aid breeders in genetic improvement of varieties nowadays. Recently, there are many ways used in crop germplasm identification as morphological, cellular, biochemical and molecular markers. They provide an effective basis for the search, identification and classification of plant crop germplasm (Erskine and Muehlbauer, 1991; Nayak *et al.,* 2005 and Li *et al.,* 2015). Praveen *et al.,* (2015) reported a Sugarcane Germplasm Database (SGDB). All sugarcane germplasm in that database are characterized by biochemical, cytological, morphological and agronomic traits including disease and insect re-

sistance. The database can improve the screening efficiency of hybrid parents greatly.

The aim of this study was to use the ISSR technique to reveal the relationship and diversity between some promising sugarcane genotypes under the Egyptian conditions compared to the commercial one. This will facilitate the selection of the genotypes that can be used in hybridization program to produce new varieties with desired traits.

MATERIAL AND METHODS

This study included six sugarcane genotypes grown under the Egyptian conditions at Sugar Crops Research Institute (SCRI) experimental station, Giza Governorate (30.022310 N, 31.207910 E). Fresh samples were delivered to Agricultural Genetic Engineering Research Institute (AGERI) to detect the differences between the different varieties using Inter Simple Sequence Repeat (ISSR) technique. The genotypes under study were G.T.54-9 (the commercial one), two new varieties G.2003-47 (G.3), G.2004-27 (G.4), two promising clones, namely G.99-103, G.2007-61 and G.84-47 variety. The pedigree of the tested sugarcane cultivars was presented in Table (1).

ISSR-PCR Reactions

A set of 11 primers ISSR (Table 2) was used in the detection of polymorphism. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgC_{l2}, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase and 30 ng template DNA.

Thermo-cycling Profile and Detection of the PCR Products

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 50ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 ug/ml) in 1X TBE buffer at 95 volts. A 100 bp DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

Data Analysis

The banding patterns generated by ISSR-PCR marker analyses were compared to determine the genetic relatedness of the samples under study. Clear and distinct amplification products were scored as ‘1’ for presence and ‘0’ for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to

Dice coefficient (Sneath and Sokal, 1973).

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where: GS_{ij} is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) as shown by Sneath and Sokal (1973).

Sample of ten stalks from three replicates were collected for the vegetative and chemical studies. The replications and mean values were compared using multiple range test (Duncan 1955) ($P<0.05$) using the computer "CoStat" statistical analysis version 6.400 described by CoHort Software (1998).

RESULTS AND DISCUSSION

Table (3) illustrated by the Figs (1-11), shows the total number of bands produced by the eleven primers used in this study. The total number of bands was 117 bands, 51 of which were monomorphic and 66 polymorphic bands with unique one (61.2 %). The highest number of polymorphic bands was 16 bands produced by primer 11 followed by primer 9, which resulted in 15 bands and primer 2, which gave 13 bands. On the other hand, the lowest number of 3 bands was produced by primer 7. Data in Table (4) reveal the unique bands with respect to the tested varieties and primers. Seven primers out of eleven *i.e.*, ISSR-2, 5, 6, 8, 11, 12 and 16 produced 17 unique bands. The primer-2 produced 3 unique bands, 2 positive ones with G.T.54-9 and G.2003-47 (G.3) varieties and a negative one with G.2007-61. Sugarcane G.84-47 variety showed 4 positive unique bands with the primers 6, 8, 12 and 16. Meantime, GT.54-9 cultivar produced three positive unique bands with the primers 2, 11 and 12. Finally, G.3 exhibited 2 bands with both primers 2 and 12. Those bands need further studies to connect them to the crop performance on either the quantity or quality.

Genetic similarity

To examine the genetic relationships among the six genotypes *i.e.*, G.T.54-9, G.2003-47 (G.3), G.2004-27 (G.4), G.99-103, G.2007-61 and G.84-47 based on ISSR results, the scored data

were analyzed using the Dice coefficient to compute the similarity matrices. These similarity matrices were used to generate a dendogram using UPGMA method. As shown in Table (5). The estimated similarities among the studied accessions ranged from 70 to 87%. The highest genetic similarity index was 87% between G.3 and G.2007-61 which is considered a good indicator for crossing between them, where the greater the similarity, the more expected the new varieties will be close to the existing excellent varieties, as mentioned by Wang *et al.* (2016). On the other hand, the lowest genetic similarity index was 70 % between G.4 and G.84-47. Referring to the pedigree of the tested genotypes (Table 1), it could be noticed that both G.T.54-9 and G.84-47 cultivars share the same female parent namely NCo.310. Therefore, the similarity between them was high (83%). The highest similarity index (87%) was that between G.3 and G.2007-61, which shared the pollen grains origin i.e, EI. (Egypt-Iran).

The UPGMA cluster analysis was carried out to detect the genetic diversity among the evaluated sugarcane cultivars and was represented graphically in Fig. (1). The dendogram (tree) obtained from UPGMA cluster analysis for the six varieties was grouped at similarity coefficient (0.79). At this level, it was divided into two main groups. The 1st group included the varieties G.84-47 and G.T.54-9 started at a distance of 0.845. Meanwhile, the 2nd group was divided into two sub groups. The 1st sub group started at a distance of 0.830, which included the two G.4 and G.99-103 genotypes. The 2nd sub group started at a distance of 0.890 and included G.3 and G.2007-61 genotypes.

The results in Table (6) cleared that the tested sugarcane genotypes differed substantially in the number of canes/m^2. Both of G.3 and G.2007-61 genotypes produced equally the highest value of this trait recording 5, 3, 2 and 1 higher number of stalks over that given by G.84-47, G.99-103, G.T.54-9 and G.4, respectively. Meanwhile, insignificant variance in stalk number/m^2 was found among G.T.54-9, G.99-103 and G.2007-61 genotypes. Moreover, except for G.84-47, insignificant difference was detected among the other genotypes in stalk number. Concerning stalk diameter, insignificant difference was noticed among the tested genotypes. The studied genotypes statistically varied in the stalk length. Sugarcane G.4 variety was the tallest among the other genotypes, where it recorded 59, 51, 47, 45 and 39 cm over that of G.3, G.84-47, G.T.54-9, G.2007-61 and G.99-103, respectively. Meanwhile, insignificant differences were found between G.T.54-9, G.2007-61 and G.84-47 in this trait. The evaluated cane genotypes varied markedly in TSS %. However, insignificant differences were recorded among G.2007-61, G.3 and G.84-47. The highest sucrose % was recorded by G.2007-61, without significant variance with other genotypes except G.99-103. The lowest value of sucrose % was recorded by G.99-103 without significant difference with G.4, G.84-47 and the commercial G.T.54-9. Sugarcane G.3

variety recorded the highest sugar recovery % without significant variance with the studied genotypes, except for G.99-103, which gave the lowest value of this quality trait. Sugarcane G.4 variety showed the appreciable superiority in cane yield with insignificant difference with G.99-103 and GT.54-9 producing 5.92, 10.91 and 11.43 ton of canes/fed over that given by G.3, G.84-47 and G.2007-61, successively. Meantime, there was insignificant difference between G.3 and the cultivated variety in the harvested cane yield/fed. The results cleared that the highest sugar yield/fed was obtained from G.4, which markedly gave 0.55, 0.86, 1.26 and 1.28 ton of sugar higher than that produced by G.3, G.T.54-9, G.99-103 and G.2007-61 genotypes without significant difference. On the other hand, insignificant difference was found in sugar yield among the commercial G.T.54-9 cultivar with G.3, G.99-103, G.2007-61 and G.84-47 genotypes.

DISCUSSION

Nowadays sugarcane breeding progress is highly focused on improvements of sugar content and abiotic stress tolerance development. The genetically diverse genotypes are being used as parentage for transferring the desirable traits to develop new sugarcane hybrids with improved traits. With respect to the environment pressures, the genomic characters of plants was not that much changed unlike to the morphological and physiological characters as claimed by Forough *et al.,* (2017). Wang *et al*., (2016) mentioned that the regional tests and integrated demonstrations can evaluate sugarcane varieties from the angle of production characteristics, where these characters include sugar yield, sugar content, disease resistance and plant height. These data are a very important reference for evaluation and promotion of sugarcane varieties. The cultivar GT.54-9 i.e. commercial variety was introduced in the Egyptian field since 1954 after passing many breeding cycles and was promoted to be the commercial cultivar at the end of seventh decade up till now. It had the most high and stable yields and good quality. Unfortunately, later it started to be infected with scale insects that threat this important cultivar. In addition to that most crops and especially sugar cane face the water scarcity problem, which made the decision makers seek for a solutions through obtaining new cultivars. Although the breeding department had succeeded to produce some promising varieties such as G.3, G.4 …and many other varieties that appeared good yields and quality parameters yet the farmer and factories still attached to the commercial one. The genetic distance between sugarcane varieties used to be determined based on the pedigree, which is dependable in most cases, it is also essential to combine the SSR molecular maker information according to Wang *et al.,* (2020). This argument is supported by Lima *et al*., (2002) who used 79 sugarcane varieties to compare genetic relationships assessed by pedigree relationship and genetic similarity coefficient based on DNA molecular maker, and they concluded that DNA molecular maker can

provide more information about genetic similarity among varieties than pedigree. In this research the variety G.2004-27 (G.4) surpassed the commercial one in many traits as stalk number, stalk length (cm), cane yield (ton/fed) and sugar yield (ton/fed). Meanwhile the similarity index between G.4 and G.99-103 was 83%, and both genotypes shared some good traits. The hybridization between them may result in a new cultivars with strong characters. Wang *et al.*, 2016 mentioned that the greater the similarity, the more expected the new varieties are to be near to the existing excellent varieties in sugar content, adaptability and yield, the more likely they are to be accepted by the growers and increase its promotion opportunities. Seventeen unique bands were obtained in our study which is surely connected to the crop behavior; however the studied traits did not appear that connection. This lead us to do further studies may be on the diseases scale or some other agronomical traits. The only negative unique band was recorded with the promising clone G.2007-61. Meanwhile, the commercial variety (G.T. 54-9) showed four unique bands and this variety is the favorite one. On the other hand, one of the promising varieties i.e. G.84-47 showed six unique positive bands, but this one recorded the least values with all studied parameters. Again further studies should be done to reveal the importance of those bands. Tazeb *et al*., (2017) concluded from their studies that genetic information obtained from the molecular-based markers can be used for establishing proper identity of the genotypes, strategic conservation of these germplasm resources, and future improvement work of the sugarcane crop through selecting the appropriate parents in their breeding programs to maximize sugar yield and maintaining genetic diversity.

CONCLUSION

ISSR as a molecular marker technique succeeded in revealing the diversity and relationships between different genotypes. These information can be used in the breeding program, for half-sib hybridization between two genetically related cane genotypes as G.3 and G.2007-61, where back crossings are usually not possible in sugar cane. Moreover, the similarity index proved that G.4 and G.84-47 varieties are genetically varied, therefore they can be crossed together to produce seeds with the highest possible diversity, which will be useful in breeding program for selection of new varieties. Further work is needed to detect the genetic relationship among genotypes to widening breeding program.

SUMMARY

Sugarcane breeding is the gate to obtain new varieties with good quality that can stand to the different changes which face that essential crop. This study was conducted to determine the relationship among six genotypes bred under the Egyptian environment using Inter Simple Sequence Repeat (ISSR) technique at Giza experimental station (30.02231^0 N, 31.20791^0 E). The six genotypes were G.T.54-9 (the commercial variety),

G.2003-47 (G.3), G.2004-27 (G.4) were new varieties, G.99-103, G.2007-61 were promising clones and G.84-47 variety. The total number of bands produced by the eleven primers, used in this study, were 117 monomorphic bands (51%) and polymorphic bands (66%), with a unique one (61.2 %). The dendogram (tree) produced from UPGMA cluster analysis for the six genotypes were grouped at similarity coefficient of 0.79. At this level, it was divided into two main groups. The 1st one included G.84-47 and GT.54-9 cultivar, started at a distance 0.845. Meanwhile, the 2nd group was divided into two sub groups. The 1st sub group started at a distance of 0.830 and included the two genotypes G.4 and G.99-103. The 2nd sub group started at a distance of 0.890 and included two genotypes G.3 and G.2007-61. The vegetative and chemical analyses emphasized the genetic relations between the studied genotypes. The G.3 and G.4 new varieties surpassed the tested genotypes in most traits under study.

REFERENCES

CoHort Software. (1998). CoStat version 6.400. 798 lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

Deren C.W. (1995). Genetic base of U.S. mainland sugarcane. Crop Sci., 35 (4):1195-1199. https://doi.org/10.2135/cropsci1995.0011183X003500040047x.

Duncan D. B. (1955). Multiple range and multiple F tests. Biometrics. 11: 1-42.

Edme S. J., Miller J. D., Glaz B.; Tai P. Y. P. and Comstock J. C. (2005). Genetic contribution to yield gains in the Florida sugarcane industry across 33 years. Crop Sci., 45 (1): 92-97. https://doi.org/10.2135/cropsci2005.0092.

Erskine W. and Muehlbauer F. J., (1991). Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theor. Appl. Gen. (1): 119-125. https://doi.org/10.1007/bf00229234.

Forough J. F., Avinash T. and Rachayya D., (2017). Analysis of Molecular Assortment in Sugarcane Varieties using RAPD and ISSR markers. Res. J. Biotech., (12): 20-28.

Hont A. D., Rao P. S., Feldmann P., Grivet L., F. N. Islam, Taylor P. and Glaszmann J. C., (1995). Identification and Characterisation of Sugarcane Intergeneric Hybrids, Saccharum officinarum × Erianthus arundinaceus, with Molecular Markers and DNA in Situ Hybridisation. Theor. Appl. Gen. (2): 320-326. doi:10.1007/BF00220894.

Jackson P. A., (2005). Breeding for improved sugar content in sugarcane. Field Crops Res., 92:277-290.

Li H., Ca S. Y., Niu J., Yuan P., Zhao D. and Zhang F., (2015). The types and application of molecular markers in the study of pomegranate germplasm resources. Acta Horticulturae 1089: 127-132. https://doi.org/10.17660/ActaHortic.2015.1089.14.

Lima M.L. A., Garcia A. A. F., Oliveira K. M., Matsuoka S., Arizono H., de Souza Jr C. L. and de Souza.A. P., (2002). Analysis of genetic similarity detected by AFLP and coefficient of parentage among genotypes of sugar cane (*saccharum* spp.). Theor. Appl. Gen. 104 (1): 30-38. https://doi.org/10.1007/s001220200003.

Nayak S., Naik P. K., Acharya L., Mukherjee A. K., Panda P. C. and Das P., (2005). Assessment of genetic diversity among 16 promising cultivars of ginger using cytological and molecular Sugar Zeitschrift fur Naturforschung C-A Journal of Biosciences. 60 (5-6): 485-492. https://doi.org/10.1515/znc2005-5-618.

Parida S. K., Sanjay K. K., Sunita K., Dalal V., Hemaprabha G., Selvi A., Pandit A., Singh A., Gaikwad K., T. Sharma R., Srivastava P. S., Singh N. K. and Mohapatra T., (2009). Informative Genomic Microsatellite Markers for Efficient Genotyping Applications in Sugarcane, Theor. Appl. Gen. 118 (2): 327-338. doi:10.1007/s00122-008-0902-4.

Praveen K., M. Hemanth Kumar, Umamaheshwari A., Reddy D. M., Sudhakar P., Munikumar M., Pradhan D. and Sabita N., (2015). SGDB: A sugarcane germplasm database. Sugar Tech 17(2): 150-155. https://doi.org/10.1007/s12355-014-0307-4.

Sneath P. H. A. and Sokal R. B., (1973). Numerical taxonomy. The principles and practice of numerical classification. San Francisco: W. H. Freeman.

Tazeb A., Haileselassie T. and Tesfaye K., (2017). Molecular characterization of introduced sugarcane genotypes in Ethiopia using inter simple sequence repeat (ISSR) molecular markers. Afr. J. Biotechnol. 16(10: 434-449. DOI: 10.5897/AJB2016. 15601

Wang Z. T., Su W. H., Que Y. X., L. Xu P., Zhang H. and Luo J.., (2016). Analysis of yield stability and test site representativeness of sugarcane trials using combined AMMI and HA-GGE biplot models. Chinese Journal of Eco-Agriculture, 24(6): 790-800. https://doi.org/10.13930/ j.cnki.cjea.151284.

Wang Z., Pan Y., Luo J., You Q., Xu L., Zhang H. Que Y., (2020). SSR-Based Genetic Identity of Sugarcane Clones and its Potential Application in Breeding and Variety Extension Sugar Tech. 22(3): 367-378.https://doi.org/ 10.1007/s12355-019-0078-9.

You Q., Pan Y. B., Xu L. P., Gao S. W., Wang Q. N., Su Y. C., Yang Y. Q., Wu Q. B., Zhou D. G. and Que Y. X., (2016). Genetic diversity analysis of sugarcane germplasm based on fluorescence-labeled simple sequence repeat markers and a capillary electrophoresis-based genotyping platform. Sugar Tech. 18(4): 380-390. https://doi.org/10.1007/s12355-015-0395-9.

Table (1): The pedigree of the tested sugarcane genotypes.

Genotype	Female ♀	x	male ♂
G.T. 54-9	NCo.310	x	F.73-925
G.2003-47	CP.55-30	x	EI.85-1697
G.2004-27	CP.55-30	x	ROC.22
G.99-103	US.74-3	x	CP.76-1055
G.2007-61	SP.73-1104	x	EI.84-2389
G.84-47	NCo.310	x	??
G.T.= Giza Taiwan, NCo. = Natal Coimbatore, F. = Formosa, CP. = Canal Point, US = United States, ROC = Republic of China, SP = São Paulo and EI. = Egypt Iran and ?? = Open pollination			

Table (2): The sequence and names of ISSR primers.

Name Primer	Sequence 5'-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR- 6	5'-CGCGATAGATAGATAGATA-3'
ISSR-7	5'-GACGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'
ISSR-9	5'-GATAGATAGATAGATAGC-3'
ISSR-10	5'-GACAGACAGACAGACAAT-3'
ISSR- 11	5'-ACACACACACACACACYA-3'
ISSR- 12	5'-ACACACACACACACACYC-3'
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR- 16	5'-TCTCTCTCTCTCTCTCA-3'
A: Adenine, T: Thymine, G: Guanine and C: Cytosine	

Table (3): Total number of bands, polymorphic, monomorphic and percentage of polymorphism as revealed by ISSR markers among six sugarcane genotypes.

Primer name	Total number of bands	polymorphic bands%	Monomorphic bands%	Polymorphism %
ISSR-2	13	5	8	38
ISSR-5	13	5	8	38
ISSR-6	9	6	3	67
ISSR-7	6	4	2	67
ISSR-8	10	4	6	40
ISSR-9	11	5	6	45
ISSR-10	15	10	5	67
ISSR-11	13	11	2	85
ISSR-12	11	6	5	55
ISSR-14	7	3	4	43
ISSR-16	9	7	2	78
Total	117	66	51	
Average	10.6	6	4.6	56.63

Table (4): The revealed unique bands base pair (bp) with respect to each genotype.

Primer name	genotype	Unique bands (bp)	
		+ ve	**-ve**
ISSR-2	G.T.54-9	1300	-
	G.2003-47	850	-
	G.2007-61	-	430
ISSR-5	G.2007-61	180	-
ISSR-6	G. 2007-61	180	-
	G.84-47	150	-
ISSR-8	G.2007-61	300	-
	G.84-47	190	-
ISSR-11	G.T.54-9	1200 & 450	-
	G.2007-61	890	-
ISSR-12	G.T.54-9	620	-
	G.2003-47	520	-
	G.84-47	330	-
ISSR-16	G.84-47	1200, 750 & 560	-

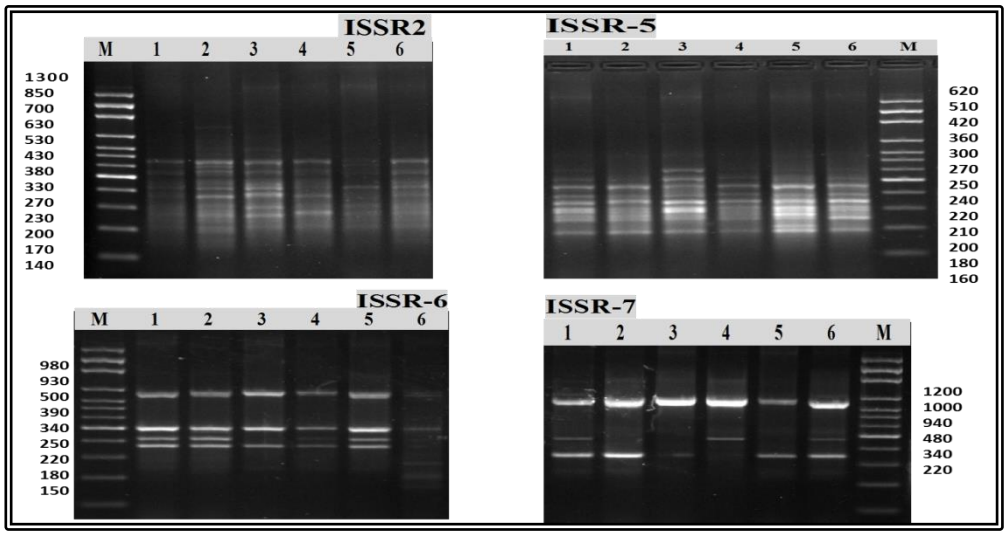
Table (5): Genetic similarity matrices among the six genotypes as computed according to Dice coefficient.

Sugarcane genotype	G.T.54-9	G.2003-47 (G.3)	G.2004-27 (G4)	G. 99-103	G.2007-61	G.84-47
G.T. 54-9	100					
G.3	83	100				
G.4	80	79	100			
G.99-103	83	84	83	100		
G.2007-61	80	***87***	78	80	100	
G.84-47	84	79	***70***	82	78	100

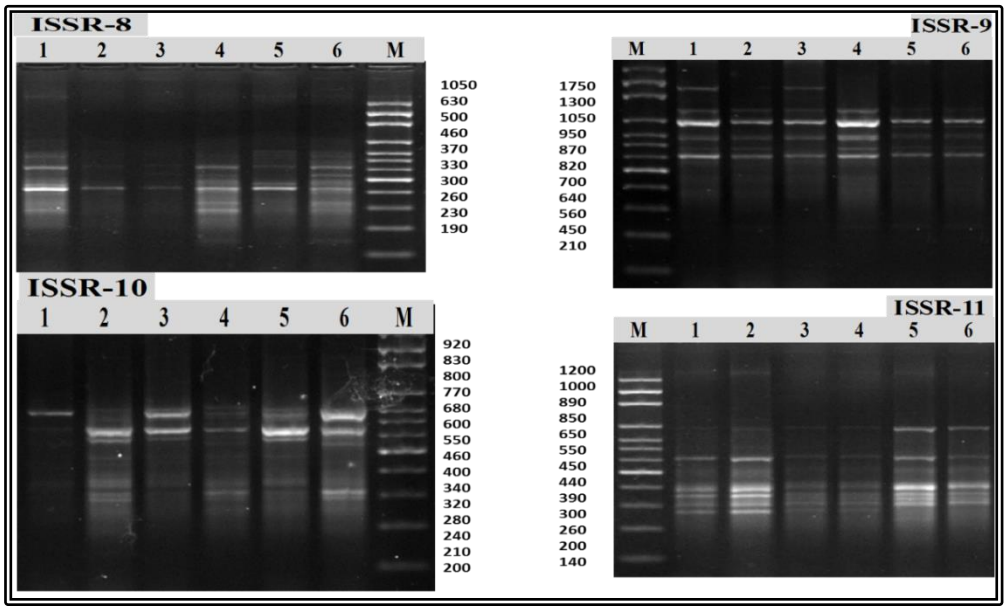
Table (6): The quantity and quality parameters for the different genotypes.

Sugarcane genotype	**Stalk Number/m^2**	**Stalk diameter (cm)**	**Stalk length (cm)**	**TSS%**	**Sucrose %**	**Sugar recovery %**	**Cane yield (ton/ha)**	**Sugar yield (ton/ha)**
G.T.54-9	10 ab	2.5 a	302 bc	18.3 c	16.16 ab	11.17 ab	58.96 ab	6.59 ab
G.2003-47 (G.3)	12 a	3.1 a	290 d	21.0 ab	17.70 a	11.96 a	57.68 b	6.90 ab
G.2004-27 (G.4)	11 a	2.9 a	349 a	18.5 bc	16.75 ab	11.72 ab	63.60 a	7.45 a
G.99-103	9 ab	3.5 a	310 b	18.6 bc	15.00 b	9.90 b	62.50 a	6.19 ab
G.2007-61	12 a	2.5 a	304 bc	21.5 a	17.72 a	11.83 ab	52.17 c	6.17 ab
G.84-47	7 b	2.4 a	298 cd	20.3 abc	16.42 ab	10.85 ab	52.69 c	5.72 b

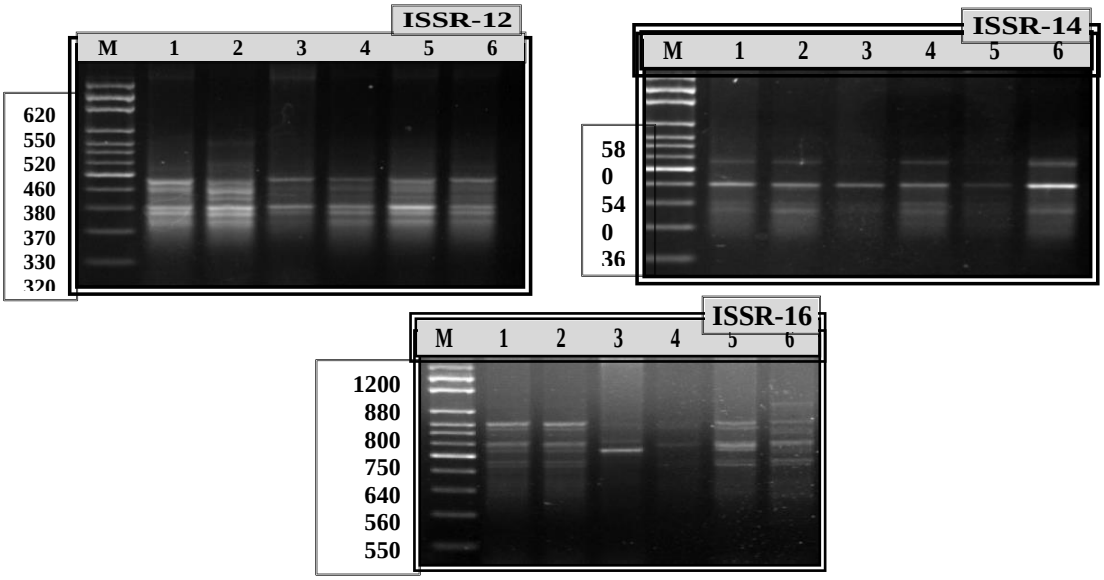
means with the same letters was not significant differe.



Figs. (1-4): Amplification profile of six sugar cane genotypes. M = Marker (1300-150) Lane 1 G.3, L2 G.4, L3 GT.54-9, L4 G.99-103, L5 G.2007-61 and L6 G.84-47.



Figs. (5-8): Amplification profile of six sugar cane genotypes. M = Marker (1750-140) Lane 1 G.3, L2 G.4, L3 GT.54-9, L4 G.99-103, L5 G.2007-61 and L6 G.84-47.



Figs. (9-11): Amplification profile of six sugar cane genotypes. M = Marker (1200-160) Lane 1 G.3, L2 G.4, L3 GT.54-9, L4 G.99-103, L5 G.2007-61 and L6 G.84-47.

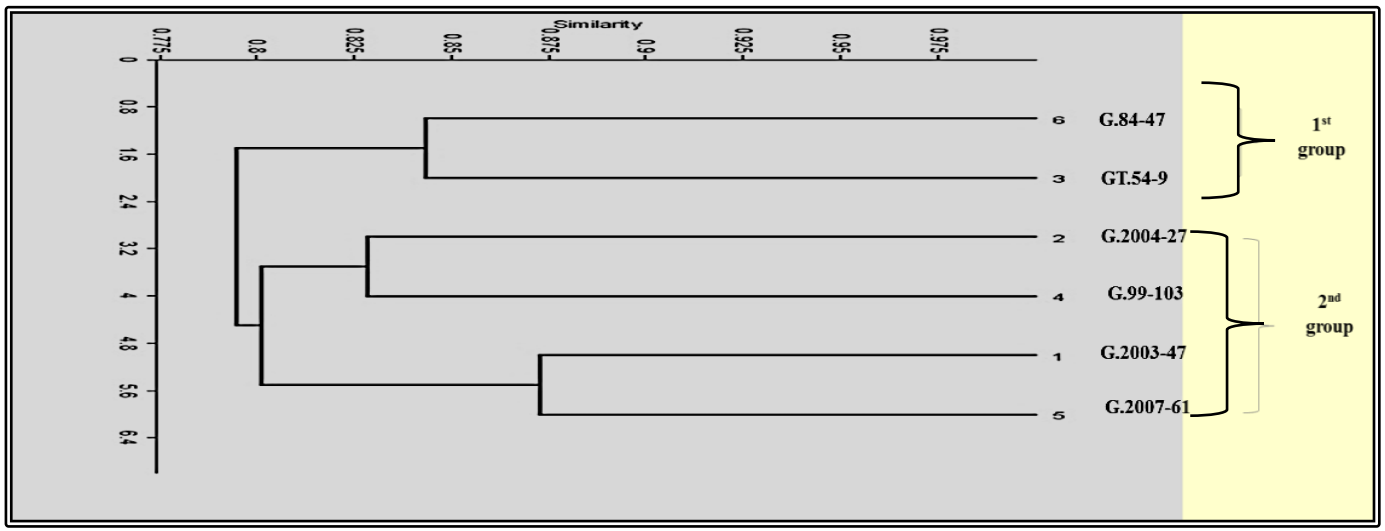


Fig. (12): Dendogram for the six sugarcane genotypes constructed from the ISSR data using Unweighted Pair-group Method using Arithmetic Average (UPGMA) and similarity matrices as computed according to Dice coefficient.