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STUDY OF GENETIC VARIABILITY IN SUGARCANE INDUCED THROUGH MUTATION BREEDING

IMTIAZ AHMED KHAN[*], M. UMAR DAHOT[**] AND ABDULLAH KHATRI

[*]*Agriculture Biotechnology Division, Nuclear Institute of Agriculture, Tandojam Sindh 70060, Pakistan,* [**]*Institute of Biotechnology and Genetic Engineering University of Sindh, Jamshoro, Pakistan.*

Abstract

Three sugarcane clones viz., NI-98, NIA-2004 and BL4 were tested for induced somatic mutation using irradiation doses of 0, 10, 20, 30, and 40Gy. The treatments 30Gy and 40Gy exhibited negative impact on the agronomic traits under study. The dose 20Gy showed stimulating and enhancing effect on plant height and cane yield (kg/plot). The analysis of variance (mean square) for all the characters under study revealed that all the radiation doses were significantly different ($P \leq 0.5$). Genetic advance at 2% selection intensity was about two fold higher than that at 30% selection intensity and intermediate at 10% selection intensity. High heritability percentage in broad sense was recorded. Variability obtained from mutation breeding was also examined through molecular marker techniques (RAPD), most similar sugarcane mutants (20 Gy) were P1 and P4 (85%) while most dissimilar mutants were P3 and Parent (38%).

Introduction

Sugarcane is a polyploid and highly heterozygous crop with wide variation in chromosome number, and is considered a difficult crop from breeding point of view. Hybridization is generally practiced under controlled environment, which is a limiting factor of many research institutes in Pakistan. Another way to obtain genetic variation is from somatic (bud) mutation either spontaneous or induced ones (Jagathesan & Sreenivasan, 1970). Induced mutation, thus play a vital role in creating additional genetic variation. Normally a large plant population is required to raise segregation population (Rao, 1969). A better way would be an efficient management of M_1 and M_2 generations that could give the greatest possibility of selection of different mutants. The use of induced mutations in sugarcane for obtaining new genetic architecture started in the early and mid of 20th century by the researchers at Hawaiian Sugar Planter's Association, Hawaii, USA. Many fruitful agronomical changes (high cane yield, high sucrose content) were recorded in the treated material (Anon., 1953). Price & Warner (1959) evaluated the prospects for sugarcane improvement by induced mutations and conceived two approaches: (i) the improvement of existing sugarcane varieties through mutagenic treatment of cuttings (ii) by incorporation of radiation technology into conventional breeding programmes.

Regarding ionizing radiations (gamma rays) in sugarcane, Tysdal (1956) observed that 4kR dose was very fatal for cane cutting (vegetative seed), while Panje & Parasad (1959) reported that 7.2 kR had little effect with approximately 50% mortality at 14.4 kR. Vijayalakashmi & Rao (1960) and Siddiqui & Javed (1982) reported that 3 kR was the safe dose to induce mutations in sugarcane. Walker & Sisodia (1969), Jagathesan & Sreenivasan (1970) and Urata & Heinz (1960) suggested that the doses between 2-4 kR were optimal for inducing mutations in sugarcane.

Very little information is available on the use of chemical mutagens in sugarcane. Hrishi *et al* (1968) observed that the effective dose for Methyl Methane Sulphonate (MMS) was 0.06M. Baroda (1987) reported that effective dose for Ethylmethane Sulphonate (EMS) and Sodium azide (SA) was $1x10^{-3}$ M and that the SA was more effective than EMS. Srivastava *et al*., (1986) indicated that 0.8% of Nitroso Methyl Urethane (NMU), Di Ethyl Sulphate (DES) and EMS were effective mutagenic agents to induced mutations in sugarcane.

Several breeders have reported the successful use of induced mutations for disease resistance in sugarcane (Jagathesan *et al*., 1974; and Srivastava *et al*., 1986), sugarcane mosaic virus (Breanx, 1975; Dermodjo, 1977) and whip (Siddiqui & Javed 1982) have been developed through mutagenesis. The present research work was conducted to estimate genetic variability obtained through the use of gamma rays induced somatic mutation for the improvement of sugarcane.

Materials and Methods

Vegetative cuttings of three sugarcane (*Saccharum* spp. Hybrid) varieties NIA-98, BL4 and NIA-2004 were irradiated with gamma rays from Cesium 137 source (Nigo 5, Bulgaria). The dose rate at the time of irradiation was 30.86Gy/minute and the treatments were 0Gy, 10Gy, 20Gy, 30Gy, and 40Gy. The irradiated material was planted in RCB design with 3 replications. The plot size was 8 x 10m, with row-to-row distance of one metre. The sowing was done in the month of September 2004 and normal agronomic practices were followed through out the growth period. Irradiated seed cuttings were grown in the field and normal agronomic practices were followed through out the growth period.

Molecular studies

Plant material: Fresh plant material of immature leaf segments was collected from 6-month-old field-grown plants RAPD analysis (Rani *et al*., 1995) was performed to confirm the genetic variability in the population developed from induced somatic mutation.

DNA extraction: DNA was extracted from fresh leaves of sugarcane plantlets using DNA isolation Kit (Gentra system, Minnesota, USA.). Two hundred mg fresh leaves were ground in liquid nitrogen; 3 ml of the cell lysis solution (Tris [hydroxymethyl] aminomethane, ethylenediaminetetra acetic acid and sodiumdodecyl sulfate) was added with leaf sample to the 15 ml centrifuge tube and incubated at 65°C for 60 minutes. Then 15µl of RNase (Gentra Kit, Minnesota, USA.) solution was added to the cell lysate and incubated at 37°C for 30 minutes. Protein precipitation solution (GENTRA Kit, Minnesota, USA.) was added and vortex for 20 seconds and the tubes were placed on ice for 30 minutes. The mixture was centrifuged at 2000 x g for 10 minutes. Supernatant containing DNA was poured in the separate 15ml centrifuge tube and DNA was precipitated by centrifuging at 2000 x g with 3 ml of isopropanol absolute. Ethanol 70% was used to wash the pellet and the DNA samples were then hydrated with TE buffer. DNA was quantified on spectrophotometer (BIOMATE 3).

Table 1. Sequence of the primers (RAPD).

Primer	Sequence	Primer	Sequence
A-01	CAGGCCCTTC	B-10	CTGCTGGGAC
A-02	TGCCGAGCTG	B-17	AGGGAACGAG
A-03	AGTCAGCCAC	C-02	GTGAGGCGTC
A-15	TTCCGAATTT	C-05	GATGACCGCC
A-18	AGGTGACCGT	C-07	GTCCCGACGA
A-20	GTTGCGATCC	C-08	TGGACCGGTG
B-06	TGCTCTGCCC	C-09	CTCACCGTCC

DNA amplification: Fourteen primers from Gene Link (NewYork, U.S.A), each ten bases in length, were used to amplify the DNA (Table 1). PCR reaction was carried out in 25µl reaction mixture containing 13ng of template (genomic DNA), 2.5mM $MgCl_2$ (Eppendorf, Hamburg, Germany), 0.33mM of each dNTPs (Eppendorf, Hamburg, Germany), 2.5U of Taq polymerase (Eppendorf, Hamburg, Germany) and 1µM of primer in a 1xPCR reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 min., at 94°C, then 32 cycles: 1 min denaturation at 94°C; 1 min., annealing at 52°C; 2 min., extension at 72°C. Final extension was carried out at 72°C for 10 min. Amplified products were analyzed through electrophoresis on 1.5% agrose gel containing 0.5X TBE (Tris Borate EDTA) at 72 Volts for 2 hours, the gel contained 0.5µg/ml Ethidium bromide to stain the DNA and photograph was taken under UV light using gel documentation system (Vilber Lourmat, France).

Data analysis: Irradiated seed cuttings were grown in the field and data recorded for nine important agronomic characters viz., plant height (cm), plant girth (cm), number of stalks per stool, weight per stool (kg), sucrose %, commercial cane sugar (CCS)%, fiber %, cane yield (t/ha) and sugar yield (t/ha). Three stools were randomly taken from each plot to determine sugar contents according to sugarcane laboratory Manual for Queensland Sugar Mills (Anon., 1970) while three rows from each plot were harvested to record yield data. The variants were assessed for genetic parameters viz., coefficient of variability, heritability percentage in broad sense and genetic advance at 2, 5, 10, 20 and 30% selection pressure. The mean and variance were computed from each treatment. Data on one plant crop and two ratoons crop was computed on above-mentioned parameters. Data was analysed by employing Duncan Multiple Range test (Steel & Torrie, 1980).

The PCR products were electrophoresed on 1.5% agarose gels using 0.5x Tris Borate EDTA (TBE) buffer and visualized by Ethidium bromide staining under UV light and photographed using Vilbour, Gel documentation System. Somaclones regenerated from each method were compared with each other using amplification profiles and band of DNA fragments were scored as presence of bands as (1) and absence of band as (0) from RAPD of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei & Li (1979). A dendrogram based on these similarity coefficients was constructed by using Unweighted Pair Group Method of Arithmetic means (UPGMA).

Results and Discussion

Field evaluation: The analysis of variance (mean square) shown in Table 2, reveals that irradiation doses were significantly different for the characters stalks/stool, plant height, sucrose%, CCS%, cane yield t/ha and sugar yield t/ha in case of BL4; plant height, cane

Table 2. Analysis of variance (mean squares) for different quantitative characters in mutated population of sugarcane.

Source of variation	D.F.	Stalk per stool (Nos.)	Plant height (cm)	Plant girth (cm)	Wt. per stool (kg)	Sucrose %	CCS %	Fibre %	Cane yield (kg/ha)	Sugar yield (kg/ha)
					BL4					
Replication (R)	2	1.439	808.889**	0.242*	0.055	6.637	6.327	0.001	1286.689**	71.058**
Treatment (T)	4	36.402**	27675.83**	0.504*	0.133*	55.941*	46.48*	1.908*	8576.144**	392.275**
R x T	8	0.814	132.500**	0.030*	0.035*	0.429	0.864	0.001	158.494**	6.767*
Year (Y)	2	2.839*	210.556**	1.803*	0.045*	3.725	3.159	3.750	398.022**	12.007**
T x Y	8	0.089	13.333**	0.003*	0.005	0.010	0.136	0.001	32.244**	1.127
Error	20	0.089	17.778**	0.073*	0.004	0.030	0.102	0.001	34.433**	0.617
					NIA-98					
Replication (R)	2	0.387	517.400**	0.001	0.001	1.330	1.813	0.194	160.422**	13.447**
Treatment (T)	4	42.127**	38250.58**	0.149*	0.137*	12.272**	15.67**	4.615**	7873.59**	223.249**
R x T	8	0.428	282.789**	0.010	0.006	0.190	0.412	0.205	21.589**	2.023*
Year (Y)	2	2.839**	96.219**	3.531	0.038	13.194**	11.61**	3.750**	125.089**	21.004**
T x Y	8	0.033	220.089**	0.004	0.001	0.496	0.492	0.001	15.506**	1.529*
Error	20	0.044	222.311**	0.005	0.001	0.287	0.506	0.001	8.689*	1.660*
					NIA-2004					
Replication (R)	2	5.342*	1055.00	0.010	0.402	0.061	0.326	0.001	45.156**	1.533
Treatment (T)	4	32.337**	16107.50**	0.092*	0.028*	1.126*	2.789*	10.792*	8887.922*	225.477**
R x T	8	1.017	155.00**	0.002	0.001	0.173	0.107	0.001	27.072**	0.745
Year (Y)	2	3.750*	375.00**	3.750*	0.713	5.078*	5.330*	3.750*	780.156**	2.726*
T x Y	8	0.001	0.001	0.001	0.001	0.423	0.481	0.001	16.072**	1.371
Error	20	0.001	0.001	0.001	0.090	0.020	0.078	0.001	25.956**	0.603

*Significance at 5% level , **Significance at 1% level

Table 3. Performance of important characteristics of sugarcane mutants developed through gamma irradiation.

Traits/treatment	Control	10Gy	20Gy	30Gy	40Gy
			BL4		
Stalk/stool (no.)	4.50c	7.00b	8.00a	3.72c	3.60c
Cane length (cm)	290a	277b	297a	205c	175d
Cane thickness (cm)	3.20a	3.10a	3.18a	2.93ab	2.63b
Cane weight (kg)	0.98a	0.98a	1.00a	0.91a	0.71b
Cane yield (t/ ha)	159.1c	175.0b	209.6a	146.1c	128.3d
Sucrose %	16.96b	16.89b	11.91c	17.66a	18.01a
CCS %	13.20ab	12.74b	8.36c	13.57ab	13.89a
Fiber %	12.58c	12.43d	13.37a	12.85b	12.16e
Sugar yield (t/ha)	21.00ab	22.38a	17.52c	19.83b	17.82c
			NIA98		
Stalk/stool (no.)	5.33c	6.43b	9.10a	4.71c	3.34c
Cane length (cm)	325b	288c	365.0a	239d	201e
Cane thickness (cm)	2.60a	2.43bc	2.55ab	2.35c	2.30c
Cane weight (kg)	0.82b	0.91a	0.95a	0.74b	0.64c
Cane yield (t/ ha)	175.7b	168.1c	208.6a	144.4d	132.4e
Sucrose %	15.42a	15.30a	12.97b	15.28a	16.00a
CCS %	10.42b	11.60ab	8.71c	11.19ab	12.09a
Fiber %	12.21b	12.98a	13.20a	11.57c	11.80c
Sugar yield (t/ha)	18.30ab	19.50a	18.11b	16.16c	16.00c
			NIA2004		
Stalk/stool (no.)	5.30b	5.83b	8.33a	4.00c	3.50c
Cane length (cm)	290b	275c	310a	245d	201e
Cane thickness (cm)	2.50b	2.59a	2.53b	2.50b	2.32c
Cane weight (kg)	0.85a	0.81a	0.92a	0.85a	0.77a
Cane yield (t/ ha)	153.7b	140.0c	184.9a	134.0c	98.33d
Sucrose %	18.33a	18.13a	17.50b	18.24a	18.30a
CCS %	14.77a	14.36a	13.30b	14.36a	14.46a
Fiber %	11.23d	12.30b	13.64a	11.34c	10.98e
Sugar yield (t/ha)	22.70b	20.08c	24.47a	19.21d	14.21e

DMR test (0.05): Means followed by the same letters are not significantly different from each other

yield (t/ha) and sugar yield (t/ha) in case of NIA-98 and stalks/stool, plant height, sucrose%, CCS%, cane yield (t/ha) and sugar yield (t/ha) in all the three genotypes. Stalks /stool were significantly ($P \leq 0.05$) higher in 20Gy followed by 10Gy in all three genotypes. Minimum stalks /stool were recorded in 40Gy in all three genotype (Table 3). Maximum plant height (cm) was observed in 20Gy 297, 365 and 310 in BL4, NIA98 and NIA 2004 respectively; whereas, minimum plant height was observed in the regenerants of 40Gy in all three genotypes. Highest cane thickness of 3.20 and 2.60 cm was observed in the untreated population of BL4 and NIA-98. In case of NIA-2004, mutants at 10Gy showed higher cane thickness as compared to control. Minimum cane thickness was

recorded in 40Gy in all three genotypes. Primary stalk weight (kg) was significantly higher in 20Gy as compared to 40Gy in BL4 and NIA-98, whereas in NIA-2004 non-significant differences were observed among the treatment and control. Minimum weight was observed in 40Gy in all three genotypes. Maximum sucrose % and CCS % was observed in 40Gy in BL4 and NIA-98 while in case of NIA-2004 no mutant could surpass the control. In case of NIA 2004 control 10Gy, 30Gy and 40Gy were statistically at par to each other. Minimum sucrose % and CCS% were recorded in 20Gy in all three genotype. Maximum value for fiber % was recorded in 20Gy in all three genotypes. Statistically significant higher cane yield (t/ha) was recorded in mutants of 20Gy and minimum cane yield was recorded in mutants of 40Gy. Highest sugar yield (t/ha) was observed in mutants of 10Gy in BL4 and NIA-98 while in case of NIA-2004 20Gy showed maximum sugar yield (t/ha).

Expected genetic advance under selection with varying selection intensities (2, 5, 10, 20 and 30%) are shown in Table 4. For any given trait, genetic advance at 2% selection intensity was almost double than that at 30% selection intensity and intermediate at 10% selection intensity. Estimates of variance components (σ^2 G x Y/y and σ^2 G x R/r) and broad sense heritability (H value in percentage) for the traits studied are given in Table 5. Heritability estimates based on three replications in each of plant cane, and first –and second –ratoon crops were relatively high for all the traits. This is not surprising since the soil type and other environmental conditions were uniform in the three replications for each crop year. Therefore, heritability estimates based on any crop year would be expected to be high also. A comparison of σ^2 G x Y and σ^2 G x R components indicated that σ^2 G x R component was larger than σ^2 G x Y in most of the traits. It indicated that this trait was less stable σ^2G x R relates to performance with in the same year, which implied that more replication should be desirable to obtain more reliable results. Whereas σ^2 G x Y component was larger than σ^2 G x R for sucrose % and CCS%, this is understandable since it is commonly observed that a ratoon cane crop has higher sucrose and CCS values as compared to plant crop.

The results indicate that the genetic variability occured in all the mutagenic treatments and agronomic traits under study showed wide range of genetic variability. Plant height and plant girth are the main contributing traits in determining cane yield (Rehman *et al.*1992; Khan *et al*. 1997). Khan *et al*. 2004 suggested that plant height and plant girth can only contribute for higher cane yield when number of stalk per stool is taken into consideration. All the mutagenic treatments showed significant difference in the stalks per stool. Singh *et al.,* (1985) reported that number of canes were the most important character contributing directly to higher yield. According to Raman *et al.*, (1985) and Javed et al., (2000), number of stalks was the major contributing factor for cane yield. Quebedeadux and Martin (1986) proposed that both the stalk number and weight should be assessed to have an accurate yield potential of the variety. Khan *et al.*, (1997) have reported that excessive stalks in stool showed adverse effect on cane yield due high intra plant competition. This may be the cause of the low yield of plantlets scored in 30GY and 40Gy. Sugar yield per unit area can be increased only if there is a break through in the production of sugarcane and the recovery of sugar. There is lack of good varieties and absence of mechanisms to carry out the package of technology and inputs to the farmers. The share of improved variety in the enhancement of cane yield and sugar recovery is about 20-25% while rest is contributed by production technology (Khan *et al.,* 2002).

Table 4. Expected genetic advance under varying selection intensities.

Traits	Expected genetic advance				
	Selection intensity %				
	2%	5%	10%	20%	30%
			BL4		
Stalk/stool (no.)	15.73	13.39	11.44	9.10	7.54
Cane length (cm)	474.18	403.64	344.85	274.31	227.29
Cane thickness (cm)	0.94	0.80	0.68	0.54	0.45
Cane weight (kg)	0.90	0.76	0.65	0.52	0.43
Cane yield (t/ ha)	214.88	182.92	156.28	124.31	103.00
Sucrose %	13.76	11.71	10.01	7.96	6.59
CCS %	14.14	12.04	10.28	8.18	6.77
Fiber %	5.23	4.45	3.80	3.02	2.51
Sugar yield (t/ha)	36.23	30.84	26.35	20.96	17.37
			NIA-98		
Stalk/stool (no.)	14.65	12.47	10.66	8.48	7.02
Cane length (cm)	402.93	342.99	293.04	233.10	193.14
Cane thickness (cm)	1.72	1.46	1.25	0.99	0.82
Cane weight (kg)	0.92	0.78	0.67	0.53	0.44
Cane yield (t/ ha)	224.88	191.43	163.55	130.10	107.79
Sucrose %	22.40	19.07	16.29	12.96	10.74
CCS %	22.00	18.73	16.00	12.73	10.54
Fiber %	3.34	2.84	2.43	1.93	1.60
Sugar yield (t/ha)	48.08	40.93	34.97	27.81	23.04
			NIA-2004		
Stalk/stool (no.)	13.83	11.77	10.06	8.00	6.63
Cane length (cm)	307.62	261.86	223.72	177.96	147.45
Cane thickness (cm)	0.73	0.62	0.53	0.42	0.35
Cane weight (kg)	0.35	0.30	0.25	0.20	0.16
Cane yield (t/ ha)	228.29	194.33	166.03	132.07	109.43
Sucrose %	7.27	6.18	5.28	4.20	3.48
CCS %	8.88	7.56	6.46	5.14	4.26
Fiber %	7.95	6.76	5.78	4.59	3.81
Sugar yield (t/ha)	36.38	30.97	26.46	21.05	17.44

RAPD studies: Amplification products in 10 Gy of the five soma clones and its parent with nine primers yielded a total of 53 scorable bands, out of which 42 (79.24%) were polymorphic and only 11(20.75%) were monomorphic (Table 4). Fragments ranged in size from 175bp-2.29kb. The number of fragments produced by various primers ranged from 3-11 with an average of 5.9 fragments per primer. The highest number of bands (11) was obtained with Primer B-10, while the lowest numbers (3) were obtained with primers B-08 and B-14 (Table 6). Amplification products in 20 Gy yielded a total of 48 scorable bands, out of which 36 (75%) were polymorphic and only 12 were monomorphic (25%). Fragments ranged in size from 192bp-2.29kb. The number of fragments produced by various primers ranged from 3-10 with an average of 5.3 fragments per primer. The highest number of bands (10) was obtained with Primer B-10, while the lowest number (3) was obtained with primer B-08 and no amplification products found in B-14.

Amplification product of 30 Gy produced multiple fragments, in which the total number of scorable bands were 50, out of which 44 (88%) were polymorphic and only 06 were monomorphic. The size of amplification products ranged from 190bp-2.29kb. The number of fragments produced by various primers ranged from 3-10 with an average of 5.6 fragments per primer. The highest number of bands (10) was obtained with Primer B-10, while the lowest number (3) was obtained with primers B-08 and B-14. Amplification product of 40 Gy produced multiple fragments, in which the total number of scorable bands were 55, out of which 38 (69.1%) were polymorphic and 17 (30.9%) were monomorphic. The size of amplification products ranged from 204bp-2.5kb. The number of fragments produced by various primers ranged from 3-11 with an average of 6.1 fragments per primer. The highest number of bands (11) was obtained with Primer B-10, while the lowest numbers (3) was obtained with primer B-14.

Table 5. Estimates of genotypic x year, genotypic x replication variances and broad-sense heritability for various traits.

Traits	σG x Y/y	σG x R/r	H%
		BL4	
Stalk/stool (no.)	0.0110	0.1420	99.98
Cane length (cm)	73.3600	94.2600	99.93
Cane thickness (cm)	0.0010	0.0030	99.63
Cane weight (kg)	0.0003	0.0020	99.99
Cane yield (t/ ha)	5.1600	7.1900	99.98
Sucrose %	0.1270	0.0360	99.99
CCS %	0.1600	0.0600	99.98
Fiber %	0.0003	0.0683	99.99
Sugar yield (t/ha)	0.5090	0.6740	99.98
		NIA-98	
Stalk/stool (no.)	0.0200	0.2700	99.97
Cane length (cm)	4.4300	44.1600	99.99
Cane thickness (cm)	0.0010	0.0100	98.44
Cane weight (kg)	0.0010	0.0110	99.69
Cane yield (t/ ha)	10.7400	52.8300	99.95
Sucrose %	0.262	0.0170	99.99
CCS %	0.257	0.0290	99.98
Fiber %	0.0003	0.0003	99.99
Sugar yield (t/ha)	0.3700	2.2500	99.98
		NIA-2004	
Stalk/stool (no.)	0.0030	0.3390	99.99
Cane length (cm)	0.0003	51.6600	99.99
Cane thickness (cm)	0.0003	0.0006	99.98
Cane weight (kg)	0.0003	0.0003	99.13
Cane yield (t/ ha)	5.3500	9.0200	99.96
Sucrose %	0.081	0.0170	99.98
CCS %	0.2930	0.0159	99.76
Fiber %	0.0003	0.0003	99.99
Sugar yield (t/ha)	0.4570	0.2480	99.97

Table 6. Primer name and amplified product obtained from irradiated population.

Primer	Range of amplified loci (bp)				Polymorphic				Monomorphic				Total no of loci			
	10 GY	20 GY	30 GY	40 GY	10 Gy	20 Gy	30 Gy	40 Gy	10 Gy	20 Gy	30 Gy	40 Gy	10 Gy	20 Gy	30 Gy	40 Gy
A-01	254-1.44kb	278-1.44kb	309-1.45kb	287-1.5kb	04	02	05	03	01	03	Nil	02	05	05	05	05
B-01	385-1.12kb	385-1.12kb	385-1.12kb	385-1.1kb	04	02	05	04	01	03	Nil	Nil	05	05	05	04
B-05	232-1.02bp	232-1.02bp	232-1.02bp	232-1.4kb	05	05	05	07	01	01	01	01	06	06	06	08
B-06	232-1.49kb	232-1.3kb	232-1.13kb	232-1.22kb	05	07	04	08	03	01	02	01	08	08	06	09
B-08	269-651bp	253-554bp	239-591bp	261-1.1kb	03	03	03	05	Nil	Nil	Nil	Nil	03	03	03	05
B-10	192-2.29kp	192-2.29kp	200-2.29kp	210-2.5kb	07	07	07	04	04	03	03	07	11	10	10	11
B-11	204-498bp	204-498bp	204-498bp	204-602bp	05	05	05	02	Nil	Nil	Nil	02	05	05	05	04
B-12	175-980bp	203-923bp	190-942bp	267-932bp	06	05	07	05	01	01	Nil	01	07	06	07	06
B-14	298-975bp	-	298-910bp	298-974bp	03	-	03	Nil	Nil	-	Nil	03	03	-	03	03
	175bp-2.29kb	**192bp-2.29kb**	**190bp-2.29kb**	**204bp-2.29kb**	**42**	**36**	**44**	**38**	**11**	**12**	**06**	**17**	**53**	**48**	**50**	**55**

Molecular markers are widely used to detect and characterize somaclonal variation at the DNA level (Ford-Lloyd *et al*., 1992). Of the available techniques, RAPD is among the most useful ones (Todoroviska *et al*., 1997). Changes in the RAPD pattern may result from the loss/gain of a primer annealing, caused by point mutations or by the insertion or deletion of sequences or transposition elements (D'Hont *et al*., 1998). The RAPD technique reveals DNA polymorphisms as differences in the amplification patterns, and uses primers of random sequences that search for complementarity in the genome. It is suggested that RAPD bands possibly represent mainly repetitive DNA (Rani *et al.*, 1995). Polymorphism in repetitive DNA sequences has frequently been observed during plant propagation by tissue culture (Smulders *et al*., 1995) and undergoes more alterations than the coding sequences (D'Hont *et al.*, 1998). *In vitro* stress may provoke changes at preferential sites, such as repetitive DNA, thereby activating transposable elements.

The similarity coefficients reflected the genetic relationship between the smaclones. The maximum similarity was observed between 10Gy and parent, and the minimum similarity was recorded between 40Gy and control in all three genotype. The dendrogram constructed on the basis of the similarity matrix showed that the mutants could be divided into three groups. Four mutants of BL4 were genetically close to each other and hence forming a group. Another distinct group is formed among mutants of NIA 2004.

Some specific bands were also identified thus, reflecting the RAPDs application for the identification of sugarcane mutants. Results revealed that at 40Gy P2 possessed a specific band of 1.459bp and band of 838bp was absent (Fig. 1).

The RAPD amplification data were used to obtain a similarity matrix and for the generation dendrogram. Similarity matrix reflects the genetic relationship between the sugarcane mutants (Table 7). The maximum similarity was obtained among P2 and P3 (93%) and minimum between P1 and Parent (71%) with 40 Gy. Maximum similarity (86%) was observed in 10 and 30 Gy with P2 and P3, while minimum similarity (33%) found in between P5 and Parent of 10 Gy. Genetically most similar sugarcane mutants (85%) were observed in 10Gy (P2 and P3) while most dissimilar mutants (40%) were observed in 20Gy (P3 and P5). High similarity between the mutant and parent reflects similarity for morphological appearance. In sugarcane RAPDs have been used to detect polymorphisms in a quick and reproducible manner (Oropeza *et al*., 1995; Nair *et al*., 1999). According to the 40 Gy denrogram, mutant cane be divided into two group, P2, P3, P4 and P1 are in group A and group B is comprises of P5 and Parrent (Dendrogram 1).

The genetic variability created in sugarcane through induced somatic mutation was efficiently assessed with molecular marker technique (RAPD). However, RAPD is a dominant marker therefore changes which may cause by the receive gene could not be identified during the screening processes. Present investigations suggested that the mutagenic doses i.e., 10Gy, 20Gy, 30Gy and 40Gy were effective in inducing genetic variability; however the irradiation dose of 20Gy showed stimulating and enhancing effect on plant height and cane yield (kg/plot). This was also confirmed by the high heritability percentage and genetic advance. For increasing per hectare sugar yield it is necessary to consider cane yield and sugar recovery togeather (Hashmi, 1995), and the genotypes be selected accordingly. Our studies show that there are possibilities of obtaining desirable mutants at intermediate dose such as 20Gy.

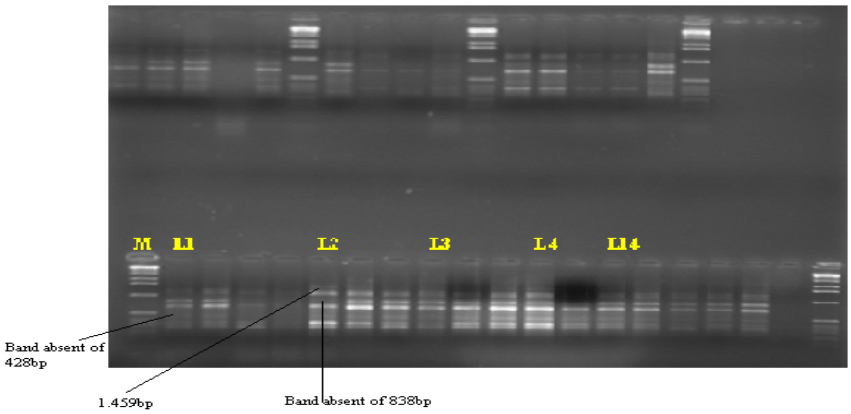
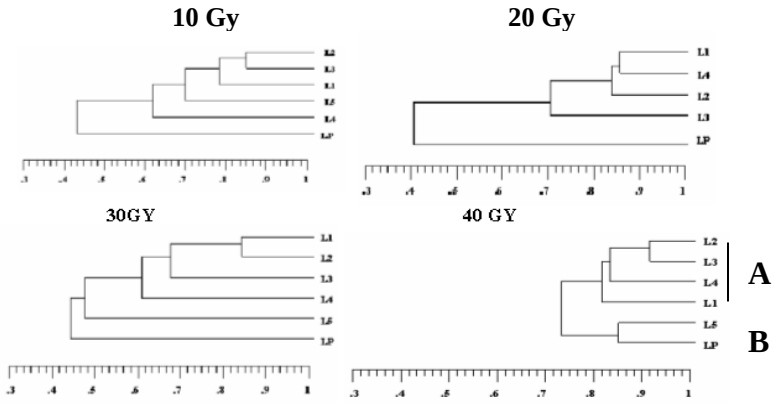


Fig. 1. Genetic polymorhism observed on gel among mutated population developed through gamma rays.



Dendrogram 1. Dendrogram of five mutants with parent developed from RAPD data using unweighted pair group method of arithmetic means (UPGMA). L1=P1, L2=P2, L3=P3, L4=P4, L5=P5, LP=Parent

Table 7. Similarity coefficient among the sugarcane mutant with parent calculated according to Ne'i & Li's coefficient.

	10 Gy					
	L1	**L2**	**L3**	**L4**	**L5**	**LP**
L1	1					
L2	0.328	1				
L3	0.836	0.859	1			
L4	0.541	0.563	0.688	1		
L5	0.711	0.647	0.752	0.657	1	
LP	0.493	0.535	0.574	0.447	0.355	1

	20 Gy				
	L1	**L2**	**L3**	**L4**	**LP**
L1	1				
L2	0.833	1			
L3	0.696	0.715	1		
L4	0.852	0.841	0.704	1	
LP	0.456	0.417	0.379	0.463	1

	20 Gy					
	L1	**L2**	**L3**	**L4**	**L5**	**LP**
L1	1					
L2	0.856	1				
L3	0.728	0.651	1			
L4	0.732	0.638	0.543	1		
L5	0.545	0.422	0.407	0.519	1	
LP	0.543	0.425	0.453	0.413	0.332	1

	30 Gy					
	L1	**L2**	**L3**	**L4**	**L5**	**LP**
L1	1					
L2	0.812	1				
L3	0.838	0.929	1			
L4	0.801	0.81	0.854	1		
L5	0.746	0.757	0.8	0.777	1	
LP	0.706	0.748	0.754	0.753	0.861	1

L1= P1, L2= P2, L3= P3, L4= P4, L5= P5, LP= Parent

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