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GENETIC VARIABILITY IN SUGARCANE PLANTLETS DEVELOPED THROUGH *IN VITRO* MUTAGENESIS

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Abstarct

Three sugarcane clones viz., NIA-98, NIA-2004 and BL4 were used for induction of genetic variability through *In-vitro* mutagenesis. Apical meristametic region was used for callus induction (4mg/l 2,4-D). Actively growing callus was treated with five different doses of gamma rays (10Gy, 20Gy, 30Gy, 40Gy and 50Gy). Maximum callus proliferation and plantlets regeneration were recorded in 20Gy and minimum at 50Gy. Maximum chlorophyll mutation frequency was noted in 40 and 50 Gy. 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) revealed significant ($P \leq 0.5$) difference for all the characters under study. Genetic advance at 2% selection intensity was about two fold greater 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

It has long been observed that the sugarcane varieties tend to run-out or decline in yield after a few years in a particular area. This demands a continuous flow of new varieties from the breeders to maintain productive genotypes in the field. The cane variety survey shows that the modern commercial canes have been derived mostly from the germplasm of 20 noble *Saccharum officinarum* and 10 *Saccharum spontaneum* species (Arcenaux, 1967; Tai *et al.,* 1994, 1995). Sugarcane improvement through plant breeding is an evolutionary process. In fact, our present day crop plants and related disciplines have made it possible to direct this evolution by utilizing hybridization, mutations and tissue culture techniques. Comprehensive improvement in this field can only be accomplished through a synergistic approach involving all the conventional and non-conventional methods of plant breeding (Siddiqui, 1994; Khatri *et al.,* 2002; Khan, *et al.*, 2000, 2002, 2004). Tissue culture when coupled with mutation breeding would become an important and valuable tool in the hands of plant breeders to create genetic variability for the selection of new genotypes with improved agronomic characteristics.

The first use of induced mutations in sugarcane has been reported in the twenties of twentieth century by the researchers at Hawaiian Sugar Planter's Association, Hawaii, USA. (Anon., 1928, 1929, 1953). The use of mutagens in combination with *In vitro* culture has also created intrest in ample plant breeders to create genetic variation. *In vitro* culture techniques offer many advantages such as expression of recessive genes that may be immediately recognized in haploid genotypes derived from the *In vitro* culture of anthers. Moreover very large population of plant cells or plants can be handled relatively

easily by *In vitro* techniques and selection for certain traits can be performed in standardized environments. Mutations occur more or less at random and for mutagenesis-derived populations, unlike segregating populations derived from cross breeding, there is no clue as to the kind or magnitude of genetic change (Brock, 1979).

As a part of biotechnology, the *In vitro* techniques offer number of advantages for mutation breeding. They may accelerate mutation breeding by rapid clonal propagation of useful mutants. The additional mutagen application can increase somaclonal variation (Maliga *et al*., 1981). Induced mutations also have useful application in term of providing marker genes for identification of fused protoplasts in somatic hybridization. (Ma1iga *et al*., 1981). Considerable work on selecting agriculturally useful somaclones has been carried out in different countries of the world such as Taiwan (Liu, 1971), Fiji (Krishnamurthi, 1974), Philippines (Lat & Lantin, 1976), Florida (Vasil *et al*., 1979), Brazil (Evans *et al*., 1980), France (Sauvarie & Galzy, 1980) and Australia (Larkin & Scowcroft, 1981). Sugarcane tissue and cell culturists have contributed significantly to the basic and applied research in this area. The present research work was conducted to estimate genetic variability obtained through the use of induced somatic mutation and biotechnological techniques for the improvement of sugarcane.

Materials and Methods

Three sugarcane (*Saccharum officinarum*) clones, NIA-2004, NIA-98 and BL4 were used for tissue culture studies. Ten explants containing leaf primordia were taken from each genotype, sterilized by standard procedure (Siddiqui *et al*., 1994) and cultured on modified MS medium (Murashige & Skoog, 1962) containing 2 mg/l and 4mg/l of 2,4-D for callusing. Media were solidified with 0.8% Difco bacto agar.

One gram of one month old callus were irradiated with 5 different doses of gamma rays (10Gy, 20Gy, 30Gy, 40Gy and 50Gy) from Cesium 137 source (Nigo 5, Bulgaria). Dose rate at the time of irradiation was 30.86 Gy/minute. Immediately after the exposure, calli were transferred on fresh media. Weight of callus cultures were recorded before and after irradiation. After five weeks of irradiation, irradiated calli were weighed and cultured on shoot induction medium (MS +2 mg/I IBA + 2 mg/I IAA + 2 mg/I kinetin). The regenerated shoots were scored for chlorophyll mutations. When the plantlets attained 7-8 cm height, these were subjected to rooting by culturing on different media viz.; i) MS medium, ii) ½ MS medium, iii) MS medium + 1mg/l IBA + 3% sugar, iv) MS +1mg/l IBA+ 4% sugar, v) MS +1mg/l IBA + 5% sugar, vi) MS + 1 mg/I IBA + 6% sugar, vii) MS + 1 mg/I IBA + 7% sugar and viii) MS + 1 mg/I IBA + 8% sugar. All these operations were carried out under aseptic conditions and cultures were incubated at 28 ± 2^{o}C with 16 hours photoperiod. Each treatment was repeated in triplicate with 50 explants per treatment. Rooted plantlets were acclimatized and transplanted in the field. The sowing was done in the month of September 2005 and normal agronomic practices were followed through out the growth period.

RAPD study

Plant material: Fresh plant material of immature leaf segments was collected from 6-month-old field-grown plants. RAPD analysis was conducted in regenerants of said clones to confirm the genetic variability in the population developed from each method.

DNA was extracted from fresh leaves of sugarcane plantlets using DNA isolation Kit (Gentra system, Minnesota, USA.) (Khan *et al*., 2007).

DNA amplification: Fourteen primers from Gene Link (NewYork, U.S.A), each 10 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), 2.5 U 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; 2min., 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 computation/analysis: Data on fresh planted crop and two ratoon crops were computed. Plantlets developed through irradiation method were grown in the field and data were recorded for 9 important agronomic characters viz., plant height (cm), plant girth (cm), number of stalks per stool, weight per stool (kg), sucrose %, commercial cane sugar (CCS)%, fibre %, 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. Statistical analysis was performed and means were compared through DMR test (Steel & Torrie, 1980). 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.

Somaclones regenerated through *In vitro* mutagenesis were compared with each other using amplification profiles. 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).

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

Results and Discussion

Callus induction: Growth response, colour and friability of the callus cultures were strongly influenced by the radiation doses (Fig. 4). Calli were initiated after 10-15 days of explant inoculation. All the varieties showed different response to callusing. Explants produced phenolic compounds, which oxidized and cause their death (Ahloowalia, 1995; Siddiqui *et al.,* 1994). The use of cystein-HCI (40 mg/1) in the medium increased the survival rate of explants (Fig. 1), as it might have prevented excessive production of polyphenolic compounds (Siddiqui, 1994). Degree of blackening of the culture medium also affects callus proliferation.

After 4 weeks of explanting the callus was sub-cultured on a fresh medium. Two types of calli were observed, yellowish white, compact, dry nodular ('A' type) (Fig. 2) and whitish globular, non-compact and wet callus type 'B' (Fig. 3). Similar type of calli was observed by Orton (1980) from tissue culture of *Hordeum vulgare, H. tubatum* and their interaspecfic hybrids. Irradiation doses of 10, 20 and 30 Gy produced 'A' type callus only whereas 40 Gy and 50 Gy doses produced both types of calluses. 'A' type callus has high potential of regeneration but 'B' type had no regeneration potential (Orton, 1980). In our study callus proliferation in BL 4 was stimulated in 10 and 20Gy treatments. Ahloowalia (1983, 1995), also reported stimulation in callus growth at low doses of gamma irradiation. However, no such stimulation was observed in NIA-98 clone. This could be due to the difference in genetic makeup of these clones.

Regeneration: Differentiation of plantlets was observed when callus tissue was transferred to regeneration medium (Fig. 5a & 5b). Regeneration was 170, 93, 114, 70, 37 and 20 in NIA-98, 165, 81, 84, 61, 37 and 15 in BL4 and 135, 80, 85, 78, 27 and 12 green plant in NIA-2004, in control, 10, 20, 30, 40 and 50Gy respectively (Table 2). Regeneration potential was directly proportional to the mutagenic treatment given to callus of each genotype but 20 Gy had stimulating effect on regeneration potential in all genotypes (Fig. 5a). Bajaj *et al.,* (1970) and Siddiqui and Javed (1982) also reported the stimulation in callus growth at low doses of gamma irradiation.

The plantlets regenerated from irradiated as well as non-irradiated callus (control) showed chlorophyll variants (Fig. 6). All clones showed maximum number of chlorophyll variants at 30 Gy, followed by 20 and 40 Gy. The low production of chlorophyll mutants in 50 Gy was probably due to less regeneration at higher dose of radiation. Siddiqui & Javed (1982) reported that 15 to 30 Gy were the optimal doses in sugarcane because growth was drastically affected by doses higher than 40 Gy. The chlorophyll variants were mostly albino and viridis (Table 3). The frequency of the chlorophyll variants was higher in NIA-2004 as compared to BL4 and NIA-98. This revealed that NIA-2004 was more sensitive to irradiation doses as compared to other genotypes. The low production of chlorophyll mutants in 50 Gy was probably due to less regeneration at higher dose of radiation. Siddiqui & Javed (1982) reported that 15 to 30Gy were the optimal doses in sugarcane and growth was drastically affected by doses higher than 40Gy. The chlorophyll variants were mostly *albino* and *viridis* and frequency of the chlorophyll variants were higher in NIA-98 as compared to BL4. This revealed that NIA-98 is more sensitive to irradiation doses as compared to BL4.

Fig. 1

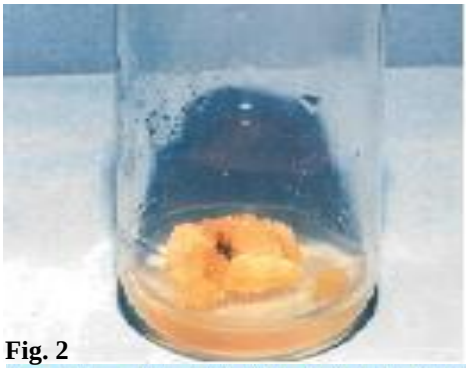


Fig. 2

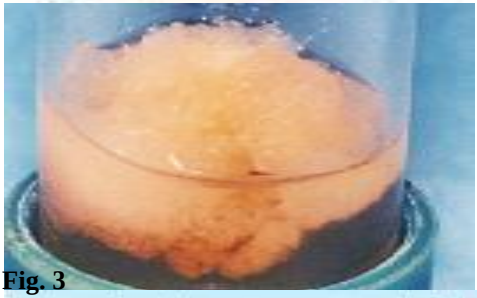


Fig. 3

Fig. 4

Fig. 5a

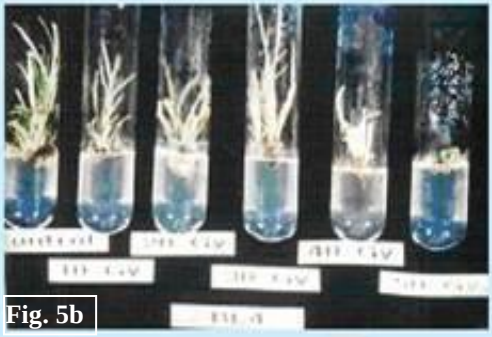


Fig. 5b

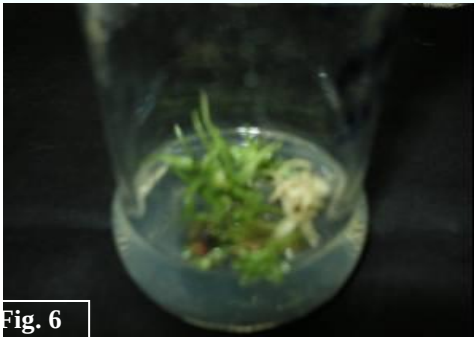


Fig. 6

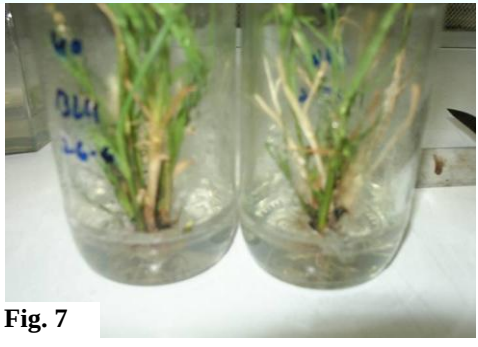


Fig. 7

Fig. 1. Explant of sugarcane containing meristametic region, Fig. 2. Globular, compact, dry regenerable callus type 'A', Fig. 3. Watery, non compact, nonglobular and non regenerable calus type 'B', Fig. 4. irradiation of explant, Fig. 5a & 5b. Shoot elongation in the irradiated callus, Fig. 6. Chlorophyll mutant and Fig. 7. Rooted shootlets of sugarcane in media containing 1mg/l IBA.

Table 3. Chlorophyll mutant obtained in regenerated plantlets of irradiated callus.

Dose (Gy)	NIA-98				BL4				NIA-2004			
	A	V	O	T	A	V	O	T	A	V	O	T
00	7	3	2	12	4	1	1	6	17	10	-	27
10	10	4	5	19	4	-	1	5	20	-	4	24
20	26	6	1	33	9	-	3	11	26	5	2	33
30	26	7	1	34	10	2	-	12	33	9	4	46
40	12	4	2	18	7	4	-	11	12	6	-	18
50	11	2	1	14	7	-	-	07	9	-	-	9

A = Albino, V = Viridis, O= Others, T= Total

Rooting: In this study problems with root initiation from callus were greater as compared with shoot initiation. Roots grow from the nodal primordia only when the plantlets are well developed. Root initiation can be obtained by qualitative and quantitative manipulation of auxins. In the present study 8 different combinations of auxins with varying levels of sucrose were used in basic MS medium for root induction. Vigorous root development (Fig. 7) was achieved when the plantlets were separated, leaves trimmed and plantlets placed on the medium containing MS + 6% sucrose + 1 mg/1 IBA. Thorpe & Biondi (1984) showed that use of IBA in medium induced vigorous root development in conifers. The plantlets with well developed shoots and roots were transferred to the jiffy pots containing sterilized perlite. After acclimatization, plantlets were first transferred to the earthen pots for hardening and then to soil.

Field evaluation: The analysis of variance (mean square) for all the characters under study is shown in Table 4. All the phytohormone concentration were significantly different at 1% level of probability for the characters stalks/stool, plant height, sucrose%, CCS%, cane yield (t/ha) and sugar yield (t/ha) in case of BL4, NIA-98 and NIA-2004.

The data regarding the average performance of all the treatments and their parents is presented in Table 5. Stalks /stool were significantly ($p \leq 0.05$) higher in 20Gy followed by 10Gy in BL4 and NIA-98, whereas in case of NIA-2004 it was maximum in 30Gy. Minimum stalks /stool were recorded in 50Gy in all three genotype. Maximum plant height was observed in 20Gy 300cm and 365cm in BL4 and NIA-98, respectively. In case of NIA-2004, maximum plant height (310cm) was observed in 30Gy. Minimum plant height was observed in the regenerants of 50Gy in all three genotypes. Highest cane thickness (cm) was observed in the control of BL4 (3.20) and NIA-98 (2.60), followed by 20Gy in both genotypes. In case of NIA-2004, 20Gy mutants showed higher cane thickness (2.59) as compared to control. Minimum cane thickness was recorded in 50Gy in all three genotype.

Primary stalk weight (kg) was significantly higher in 20Gy (1.11, 0.95) and in 30Gy (0.82) as compared to control, in BL4, NIA-98 and NIA-2004, respectively. Minimum weight was observed in 50Gy in all three genotype. Maximum sucrose % and CCS % was observed in 50Gy in BL4 and NIA-98 while in case of NIA-2004, no mutant could surpass the control. In NIA-2004, control, 10Gy and 50Gy were statistically at par to each other. Minimum sucrose % and CCS % were recorded in 20Gy in all three genotypes. Maximum value for fiber % was recorded in 20Gy in all three genotypes. Statistically significant higher cane yield was recorded in mutants of 20Gy in BL4 and NIA-98 while in case of NIA-2004 it was higher in 30Gy. Minimum cane yield was recorded in mutants of 50Gy. Highest sugar yield (t/ha) was observed in mutants of 10Gy in BL4 and in control in NIA-98 and NIA-2004.

Table 5. Pooled performance of important characteristics of sugarcanesomaclones developed through in vitro mutagenesis.

Traits/treatment	Control	10Gy	20Gy	30Gy	40Gy	50Gy
			BL4			
Stalk/stool (no.)	3.60d	7.00b	8.00a	4.50c	3.27d	2.08e
Cane length (cm)	290.0ab	280.0b	300.0a	203.9c	175.0d	105.0e
Cane thickness (cm)	3.20a	3.10ab	3.16ab	2.93b	2.63c	2.23d
Cane weight (kg)	0.98b	0.98b	1.11a	0.92b	0.71c	0.57d
Cane yield (t/ ha)	156.8c	205.1b	232.3a	139.1d	118.8e	75.00f
Sucrose (%)	17.17b	16.94b	11.20c	17.62ab	11.88c	18.07a
CCS (%)	13.18ab	12.86b	6.95	13.57ab	8.32c	13.97a
Fiber (%)	12.58d	12.16f	13.86a	12.85c	13.37b	12.43e
Sugar yield (t/ha)	20.67c	26.40b	16.14c	18.87c	9.88d	10.47e
			NIA 98			
Stalk/stool (no.)	5.11c	6.43 b	9.10a	4.60 c	3.23 d	2.00 e
Cane length (cm)	325 b	300 c	365 a	240 d	201 e	121.7 f
Cane thickness (cm)	2.60 a	2.43 b	2.56 a	2.30 c	2.3 c	1.87 d
Cane weight (kg)	0.82 b	0.91 a	0.95 a	0.74 c	0.64 d	0.44 e
Cane yield (t/ ha)	192.8 b	163.70 c	242 a	156.9 c	116.20 d	75.53 e
Sucrose %	15.63 b	15.35 b	11.26d	15.55 b	13.36 c	16.23 a
CCS %	10.63 c	11.52 b	7.08e	11.43 b	8.91 d	12.23 a
Fiber %	12.68 b	12.98 ab	13.20a	11.57 d	12.21 c	11.80d
Sugar yield (t/ha)	20.50a	18.81b	17.13b	17.92 b	10.35 c	9.23d
			NIA2004			
Stalk/stool (no.)	4.00c	3.50c	5.61b	8.33a	5.30b	1.79d
Cane length (cm)	201.7e	290.0b	275.0c	310.0a	245.0d	114.0f
Cane thickness (cm)	2.32b	2.50a	2.59a	2.54a	2.50a	2.00c
Cane weight (kg)	0.67e	0.75c	0.71d	0.82a	0.75b	0.31f
Cane yield (t/ ha)	120.7d	157.1b	157.4b	202.7a	139.0c	75.33e
Sucrose %	18.44a	18.12a	15.79c	18.01ab	17.50b	18.37a
CCS %	14.87a	14.32ab	11.52d	13.81bc	13.21c	14.39ab
Fiber %	11.32c	12.30b	13.65a	11.34c	13.64a	10.98d
Sugar yield (t/ha)	23.37a	22.52a	23.35a	19.20b	15.93c	10.79d

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

Expected genetic advance under selection with varying selection intensities (2, 5, 10, 20 and 30%) are shown in Table 6. For any given trait, genetic advance at 2% selection intensity was about 2 times greater 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 7. Hertibility estimates based on three replications in each of plant cane, and first and second ratoon crops were relatively high for all the traits. A comparison of σ^2 G x Y and σ^2 G x R components indicated that ^{2} σG x R component was larger than σ^2 G x Y in most of the traits. Which 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 ^{2} σG 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.

Table 6. Expected genetic advance under varying selection intensities.

Traits	Expected genetic advance				
	Selection intensity %				
	2%	5%	10%	20%	30%
			BL4		
Stalk/stool (no.)	16.68	14.20	12.13	9.65	7.99
Cane length (cm)	564.754	480.741	410.730	326.717	270.709
Cane thickness (cm)	2.75	2.34	2.00	1.59	1.32
Cane weight (kg)	1.46	1.24	1.06	0.84	0.70
Cane yield (t/ ha)	416.835	354.827	303.153	241.144	199.805
Sucrose %	18.12	15.42	13.18	10.48	8.68
CCS %	21.20	18.05	15.42	12.26	10.16
Fiber %	4.60	3.92	3.35	2.66	2.20
Sugar yield (t/ha)	51.19	43.57	37.23	29.16	24.53
			NIA-98		
Stalk/stool (no.)	18.13	15.43	13.19	10.49	8.69
Cane length (cm)	647.710	551.357	471.062	374.700	310.470
Cane thickness (cm)	1.91	1.63	1.39	1.10	0.91
Cane weight (kg)	1.37	1.17	1.00	0.79	0.66
Cane yield (t/ ha)	423.458	360.464	307.969	244.975	202.979
Sucrose %	8.54	7.27	6.21	4.94	4.09
CCS %	9.65	8.21	7.02	5.58	4.62
Fiber %	4.79	4.07	3.48	2.77	2.29
Sugar yield (t/ha)	61.32	52.20	44.60	35.48	29.39
			NIA-2004		
Stalk/stool (no.)	16.21	13.80	11.79	9.38	7.77
Cane length (cm)	523.821	445.897	380.961	303.037	251.088
Cane thickness (cm)	1.58	1.34	1.14	0.91	0.75
Cane weight (kg)	1.31	1.12	0.95	0.76	0.63
Cane yield (t/ ha)	309.524	263.479	225.108	179.063	148.367
Sucrose %	2.78	2.36	2.02	1.60	1.33
CCS %	4.17	3.55	3.03	2.41	2.00
Fiber %	8.80	7.49	6.40	5.09	4.22
Sugar yield (t/ha)	47.35	40.30	34.43	27.39	22.69

The results indicate that the genetic variability occured in all the mutagenic treatments and both quantitative and qualitative traits showed wide range of genetic variability. The plant height and cane girth are the major contributing factors for high cane yield (Rehman *et al*., 1992; Khan *et al*., 1997, 1998, 1999). Khan *et al*., 2004 also reported that plant height and plant girth are the important cane yield contributor along with stalk per stool. Singh *et al*., (1985) have reported that number of cane 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 & Martin (1986) proposed that both the stalk number and weight should be assessed to have an accurate yield potential of the variety. In contrast Khan *et al*., (1997) have reported that excessive stalks in stool showed adverse effect on cane yield to due higher intra plant competition. Sugar yield per unit area can only be increased if yield and recovery of sugar go together, which is very rare. The potential of any high yielding variety can only be achieved when proper production technology is adopted. 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 7. 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.)	0.009	0.266	99.99
Cane length (cm)	0.1543	68.673	99.99
Cane thickness (cm)	0.0043	0.0106	99.86
Cane weight (kg)	0.0003	0.0020	99.96
Cane yield (t/ ha)	8.09	37.64	99.99
Sucrose %	0.143	0.003	99.99
CCS %	0.288	0.045	99.98
Fiber %	0.0003	0.0003	99.99
Sugar yield (t/ha)	1.04	1.14	99.98
	NIA-98		
Stalk/stool (no.)	0.006	0.1533	99.99
Cane length (cm)	0.0003	29.389	99.99
Cane thickness (cm)	0.0003	0.0026	99.98
Cane weight (kg)	0.0003	0.0016	99.96
Cane yield (t/ ha)	13.58	161.06	99.98
Sucrose %	0.165	0.063	99.74
CCS %	0.164	0.137	99.64
Fiber %	0.0003	0.0580	99.99
Sugar yield (t/ha)	1.04	1.14	99.99
	NIA-2004		
Stalk/stool (no.)	0.008	0.421	99.99
Cane length (cm)	0.0003	66.72	99.99
Cane thickness (cm)	0.0003	0.0023	99.99
Cane weight (kg)	0.0003	0.0003	99.83
Cane yield (t/ ha)	7.95	51.68	99.99
Sucrose %	0.141	0.057	99.83
CCS %	0.160	0.035	99.71
Fiber %	0.0003	0.0050	99.99
Sugar yield (t/ha)	0.35	2.36	85.52

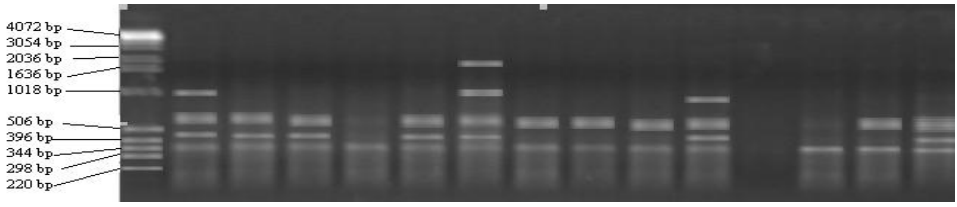
RAPD studies: Genomic DNAs of the 13 somaclones and its parent produced multiple fragments with 14 arbitrary primers out of 23 primers. The total number of scorable bands were 52, of which 17 (32.69%) were polymorphic and 35 (67.31%) were monomorphic. The number of fragments produced by various primers ranged from 1- 6, with an average of 3.5 fragments per primer. The level of polymorphism varied with different primers (Gel 1 & 2). Maximum 6 bands were amplified with primer A-02 and A-20 while minimum one band was amplified with primer A-03, C-09 and B-05. The size of fragments ranged from 230 bp- 3.85 kbp.

Molecular markers are widely used to detect and characterize somaclonal variation at the DNA level (Ford-Lloyd *et al*., 1992; Cloutier & Landry, 1994; Barrett *et al*., 1997). Of the available techniques, RAPD is among the most useful ones (Sterck & de Vries, 1993; Rani *et al*., 1995; Taylor *et al*., 1995; Shoyama *et al*., 1997; Todoroviska *et al*., 1997; Rout *et al*., 1998). 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

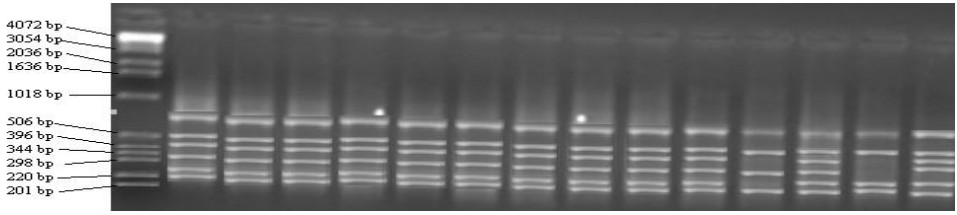
or transposition elements (Peschke *et al*., 1991). The RAPD technique reveals DNA polymorphisms as differences in the amplification patterns, and primers of random sequences that search for complementarity in the genome. It is suggested that RAPD bands possibly represent mainly repetitive DNA (Grattapaglia & Sederoff, 1994). Polymorphism in repetitive DNA sequences has frequently been observed during plant propagation by tissue culture (Smulders *et al*., 1995, 2005) and undergoes more alterations than the coding sequences. *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 clones. The maximum similarity was observed between somaclones 01, 02, 03 (98%) and 07, 08 and 09 and the minimum similarity was recorded between somaclone 05 and 13 (Table 8). The dendrogram constructed on the basis of the similarity matrix showed that the somaclone 01, 02 and 03 were identical but distinct from its parent. Somaclone 08, 09, 07, 10, 06 and 05 were more closely related to each other and forming a separate group in the dendrogram. Another group was formed between somaclone 11, 12 and 13 (Dendrgram 1).

The genetic variability created in sugarcane through *In vitro* mutagenesis was efficiently assessed with molecular marker technique (RAPD). However, RAPD is a dominant marker therefore changes which may cause by the gene could not be identified during the screening processes. Present investigations suggested that the mutagenic doses i.e., 10Gy, 20Gy, 30Gy, 40Gy and 50Gy 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.



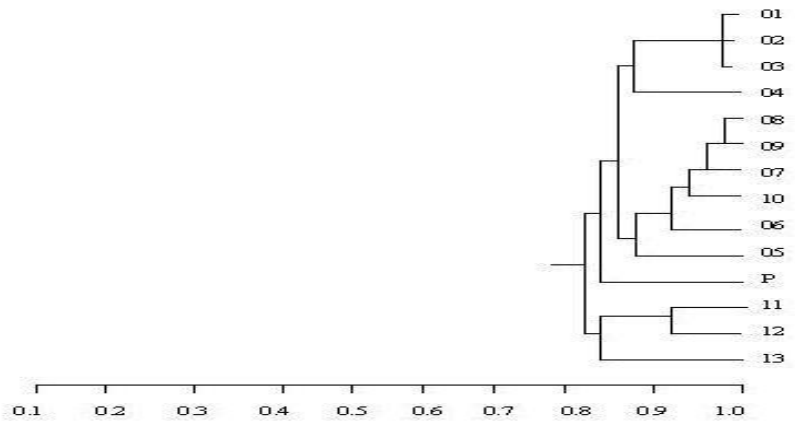
Gel 1. Genetic polymorphism observed in somaclones regenerated through *In vitro* mutagenesis with Primer A-18.



Gel 2. Genetic polymorphism observed in somaclones regenerated through *In vitro* mutagenesis with Primer C-07.

Table 8. Similarity coefficient among the sugarcane somaclones developed through *In vitro* mutagenesis calculated According to Nei & Li (1979).

	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**Parent**
01	1.00													
02	**0.98**	1.00												
03	**0.98**	0.96	1.00											
04	0.89	0.91	0.87	1.00										
05	0.87	0.89	0.89	0.87	1.00									
06	0.87	0.85	0.89	0.83	0.89	1.00								
07	0.89	0.91	0.91	0.89	0.91	0.94	1.00							
08	0.92	0.91	0.94	0.89	0.91	0.94	0.96	1.00						
09	0.91	0.92	0.92	0.91	0.92	0.92	**0.98**	**0.98**	1.00					
10	0.87	0.89	0.89	0.87	0.89	0.89	0.94	0.94	0.96	1.00				
11	0.79	0.77	0.81	0.79	0.89	0.89	0.87	0.87	0.85	0.89	1.00			
12	0.79	0.81	0.81	0.87	0.85	0.85	0.91	0.87	0.89	0.92	0.92	1.00		
13	0.75	0.74	0.77	0.79	0.70	0.81	0.79	0.79	0.77	0.81	0.81	0.85	1.00	
Parent	0.87	0.89	0.89	0.79	0.89	0.81	0.87	0.87	0.89	0.89	0.81	0.81	0.74	1.00



Dendrogram 1. Genetic similarity among the somaclones regenerated through *In vitro* mutagenesis.

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