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INDUCED MUTATIONS AND SOMACLONAL VARIATIONS IN THREE SUGARCANE (*SACCHARUM OFFICINARUM* L.) VARIETIES

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Abstract

Sugarcane is an important field crop of tropics and sub-tropics. Three sugarcane varieties viz. NIA-0819, NIA-98 and BL4 were subjected to induced mutations by using four different doses of gamma radiation (10, 20, 30 and 40Gy).Data on various parameters were collected including auricle, legule, stalk colour, bud shape, number of tillers plant^{-1}, number of internodes, stool weight, cane yield, brix %, purity %, commercial cane sugar (CCS %), sucrose %, and sugar yield. Significant differences were observed for most of the traits in the study. The maximum tillers plant^{-1} was recorded in NIA-98 at 20Gy and the lowest number of tillers plant^{-1} was seen in BL4 at 30Gy. Best stool girth was observed at 20Gy in NIA-98. However, longer length leaf was obtained in NIA-0819 at 20Gy, while the short leaf length was recorded in BL4at 40Gy. The maximum brix % was observed in BL4 at 30 and 40Gy. Commercial cane sugar percentage was highest at 10Gy in NIA98. The maximum sugar yields were obtained at 10Gy in NIA-98 whereas; the minimum sugar yield was recorded at 40Gy in BL4. Highest cane yield was achieved at 10Gy in NIA-0819, followed by 20Gy in NIA-98. The gamma radiation doses of 30 and 40Gy showed negative effect on the cane yield in all varieties. Thus, somaclones of NIA-0819at 20Gy and NIA-98 at 10Gy showed the best performance in respect of cane yield, sugar yield and juice quality. Cluster analysis divided the genotypes into four major groups. The cluster I was distinguished by its highest sucrose%, sugar yield, and leaf width values. Whereas, cluster II was observed to be unique in terms of its highest plant height, internodes length, leaf length, CCS% and purity %. Moreover, cluster III represented the group of genotypes having maximum quantitative traits (plant height, number of tiller/plant, stool weight, girth, number of internodes, internodes length and cane yield) coupled with low qualitative observations. Furthermore, cluster IV encompassing five genotypes, showed excellent qualitative characteristics along with low quantitative traits. Hybridizing the genotypes from different clusters of the analysis can be of promising outcomes in terms of getting the genetic diversity among progeny plants for further evaluation in cane breeding programs.

Key words: Sugarcane, Gamma radiation doses, Agronomic traits, Cluster analysis.

Introduction

Sugarcane is a member of the genus *Saccharum*, the most important crop for sugar production. The *Saccharum* species are usually highly polyploid with no known diploids. Interspecific variability of chromosome number ranges from 80 to 120 and maintenance of aneuploids are characteristics of sugarcane (Sreenivasan *et al*., 1987 and D'Hont *et al.,* 1998). Most of the commercial sugarcane varieties are now in use of descendant of inter-specific hybrids within the genus *Saccharum* (Dillon *et al*., 2007).

The highest sugarcane producing countries are Brazil, India, China, Thailand and Pakistan (FNP 2009). Average production of sugarcane in different countries of the world is about, 100 tha^{-1} while in Pakistan it is only 55 tha^{-1}(Anon, 2015). Similarly, sugar recovery of Pakistan floats below 9.7%, which is the cause of high cost in sugar production (PSMA, 2012). In spite of being fifth largest grower of the crop with respect to area under cultivation, the productivity per unit area in Pakistan is one of the lowest among all sugarcane growing countries. Although, there are numerous reasons of low yields of the crop in the country however, non-availability of high sugar yielding clones and poor production technology is the most impacting factors to count (Ali *et al*., 2008; Khan *et al.,* 2015). Conventional sugarcane breeding in Pakistan is hampered by environmental as well as genetic barriers. The induced mutations can therefore have a vital role in improving sugarcane since it is propagated vegetatively in the country.

The first use of induced mutations for sugarcane improvement was carried out at Hawaiian sugar planter's Association, Hawaii, USA (Anon., 1928). Several scientists have earlier reported that 20Gy is the best gamma radiation dose for sugarcane crop (Khatri *et al.,* 2002:, Khan *et al.,* 2004and Ikram *et al.,* 2010.On the other hand different scientists reported that the higher doses of gamma radiation produced generally negative effects on plant growth and development; although the effect of gamma radiation dose on mutation frequency power differs among plant species (Ali *et al*., 2016).

Mutation breeding is one of the basic techniques used in crop improvement (Oladosu *et al*., 2016 and Novak *et al.,* 1992).The agronomical parameters like high sucrose percentage and high cane yield t ha^{-1}have been noted in the mutant material (Khan *et al.,* 2004 and Hussain *et al*., 2005).The mutation breeding as an effective technique to enhance of existing sugarcane variations through mutagenic doses. Siddique *et al*. (1994) reported that, it can induce useful as well as harmful influence in crop plants. Thus there was a need to find out the most beneficial dose of gamma radiation for the improvement of growth and yield parameters in sugarcane. Many breeders have reported the fruitful use of induced mutations used for disease resistance in sugarcane (Ramana *et al.,* 2001). Resistance for mosaic virus, and whip smut of sugarcane have been established through induced mutagenesis (Esh *et al.,* 2014; Ganesh *et al.,* 2015). The genetic diversity associated between populations and investigation of

dissimilar groups of specific lines of sugarcane that can be excellently explored using of cluster analysis (Ilyas, 2011, Klomsa *et al.,* 2013, Brasileiro *et al.*, 2013, Tahir *et al.,* 2013, You *et al.,* 2013). Such disectional techniques have been used in several investigations to get insight into huge data collected from agronomic evaluations of the different crops viz. cotton and bread wheat (Ogunbayo *et al.,* 2005, Rana *et al.,* 2005, Khodadadi *et al.,* 2011, Fahim 2014). In the present study field performance of mutant of sugarcane varieties was evaluated with the aim to score genetic variation created by gamma radiation. It is expected that the results of such studies will ultimately be helpful in developing of sugarcane clones for commercial release.

Materials and Methods

Three sugarcane varieties viz. NIA-98, NIA-0819 and BL4 were used for genetic variability, through gamma radiation. Four different doses of gamma radiation doses (10, 20, 30 and 40Gy)were used through(CO^{60}) gamma source (Model-Theratron-780) from Nuclear Institute of Medical and Radiotherapy (NIMRA), Jamshoro, Pakistan at room temperature (22-25^{0}C) (Ping *et al*., 1999)and untreated material were used as control, the dose rate at the time of radiation was 58 seconds per Gy. After established plantlets were developed from gamma radiation doses were transferred in to field for further screening and evaluation. The experiment was laid out in randomized complete block design with three replications. Each replication consisted of 10 x 10 m plot, with a row to row distance of one meter, to evaluate the incidence of mutation data of different qualitative and quantitative parameters were recorded after 10-12 months of planting.

Morphological parameters: The morphological parameters viz. shoot habit, tillering habit, tillering density, auricle, leaf length, legule, stalk colour; bud shape, stalk height, tillersplant^{-1}, internodes length, stalk weight, cane yield and sugar yield were taken after the age of 10-12 months of sugarcane crop.

Biochemical parameters: The biochemical parameters viz. brix %, CCS %, purity %, juice quantity, sugar recovery % and fiber % were taken five plants randomly from each replication. Sugar contents were analyzed according to "Sugarcane Laboratory Manual for Queensland Sugar Mills" (Anon.1970) and yield data was recorded as Khan *et al.* (2009).

Statistical analysis: The experimental data were recorded and subjected to randomized complete block design with factorial arrangement of analysis of variance (ANOVA) under linear models of statistics to observe statistical differences among different traits of sugarcane by using computer program, Student Edition of Statistix (SWX), Version 8.1 (Analytical Software, 2005). Further least significant difference (LSD) test was also applied to test the level of significance among different combination means (Gomez and Gomez, 1984).

Result and Discussion

Field evaluation qualitative and quantitative traits in sugarcane: Analysis of variance (mean square), as presented in Tables 1-2, showed that the gamma radiation doses showed statistically significant differences for different characters including number of internodes, stool weight, cane yield, brix %, purity %, CCS %, sucrose % and sugar yield and cane yield.

Field evaluation of sugarcane variety NIA-0819 showed maximum plant height (457 cm) under control, whereas, in BL4 plant height was reduced (131 cm) at 20Gy. The numbers of tillers are major yield contributing trait in sugarcane (Yasmin *et al*., 2011). The maximum number of tillers plant^{-1} were observed in NIA-0819 at 20 and 30Gy (11.00), followed by (10.00) in NIA-98 at 20Gy and lowest number of tillers plant^{-1} were recorded (5.00) in BL4 at 30 and 40Gy respectively. On the other hand, maximum stool weight was observed (10.16) in NIA-98 and NIA-0819 (10.00) at 20Gy, while, minimum stool weight was recorded (5.00) at 40Gy in BL4.

The maximum number of internodes were observed (29.66) in NIA-98 at 10Gy, stool girth (2.46 cm) in NIA-98 at 20Gy.Similar results were obtained by Doule *et al.* (2008) and Dalvi *et al.* (2012) in a field study sugarcane mutant's plant and they also observed significant variations for the characters like number of tillers plant^{-1}, stalk length, and stalk diameter. Similarly result of mutagenesis for greater length of internodes, and smaller cane diameter was reported by Sood *et al.,* 2006.

Regarding qualitative parameters of sugarcane varieties showed that maximum brix (19.06 and 18.98%) in BL4 at 30 and 40Gare presented in Table 3. Moreover, maximum purity (75.78%), CCS (8.32%) was observed at 10Gy in NIA-98, while higher sucrose (14.13%) was observed under control in NIA-98. Furthermore, it was noted that the highest fiber (13.18%) was recorded in BL4 at 40Gy. Cane and sugar yield are the most important parameters of sugarcane crop. The highest cane yield was achieved (101.67 t ha^{-1}) in NIA-0819 at 20Gy, followed by (100.00 t ha^{-1}) in NIA-98 at 10Gy. The maximum sugar yield was recorded (8.42 t ha^{-1}) in NIA-98 at 10Gy, while lowest sugar yield was achieved (3.63 t ha^{-1}) in BL4 at 40Gy. Several earlier studies have shown similar results regarding the role and efficiency of gamma radiations for generating variations in sugarcane characteristics. Khan *et al.* (2015) and Doule *et al*. (2008) reported that high brix %, sucrose %, CCS % and sugar recovery % in progeny plants against their parent. It was seen in this study also that the mutated plants surpassed the parent in many of the qualitative as well quantitative parameters. However, contrarily, Khan *et al.* (2004) reported that brix % declined in progeny plants against the parent, which shows that gamma radiation doses are expected to produce versatile results. Some of the other publications, including that of Khan *et al.* (2004), and Jain, (2000) have also narrated that that induced mutations can be excellently employed for the purpose of gaining genetic diversity and better agronomic characteristics in progeny plants. Such efforts have been exploited by numerous scientists to recover improved plantlets from a mother genotype. This signifies the importance of radiation mutagenesis for high yielding cultivars. Mutation breeding has been employed for enhancement of cane yield, sucrose percentage and resistance to pests and diseases (Cox *et al.,* 1996, Srivastiva

et al., 1986). The promising mutants from such experiments can be further evaluated as new genotypes, and may also be utilized in breeding programmes as genetic stock to improve the yield and qualitative traits in sugarcane.

Morphological parameters: The morphological data also revealed significant variations in the mutant plants as compare to their parent (Tables 4, 5 and 6), which denotes the significant degree of variations which can be used for the creation of genetic variability in sugarcane (Khan *et al.,* 2009).

Morphological character of leaf: Longest leaf length was noted in the variety NIA-98 (180 cm) at 20Gy and shortest leaf length (117 cm) was measured in BL4 at 40Gy. At maturity, minimum numbers of green leaves were found in BL4 at 30Gy and 40Gy, while highest number was noted in the variety NIA-0819. Presence of green leaves also illustrate the maturity pattern in poaceae family. Higher number of green leaves means late maturity. The data, as in Table 5, showed 33% variation in the green leaves in mutagens of BL4 as compared to the parent. The maximum leaf width was observed in NIA-0819 (5.30 cm) at 20Gy and minimum leaf width in BL4 (3.33 cm) at 40Gy. Most of the varieties had medium leaf lamina in all the treatments. 80% variations were recorded in sheath colour in the treatments of BL4, while for NIA-0819, and NIA-98 the variation percentage was 90% and 95% respectively. Moreover, in case of BL4 plant growth habit was observed to be semi erect whereas mutagens exhibited erect plant standing. Degree of dissimilarity of mutants as compared to the parent in NIA-0819, BL4 and NIA-98 were observed to be 35%, 35%, and 22% respectively (Tables 4, 5 & 6).

Characteristics of the cane stalk: Variation in stalk colour was observed at 20, 30 and 40Gy whereas no variation was recorded at 10Gy. The variation was 40%, 30% and 20% in NIA-98, BL4 and NIA-0819, respectively. Moreover, high tillering density was monitored in 20%, 24% and 16% plants of NIA-98, BL4 and NIA-0819, respectively. In case of stalk thickness, thick to medium stalks were observed in 10 and 20Gy of all the treatments and thin diameter stalks were noticed at 30Gy and 40Gy. Furthermore, variation in internodes shape was 24%, 35% and 25% in NIA-0819, BL4 and NIA-98, respectively (Figs. 4-7). Bud shapes of the progeny also showed variation by as high as 25%, 20% and 30% in NIA-0819, BL4and NIA-98, respectively. Bud colour, bud groove, and bud size also exhibited significant differences (Tables 4, 5 & 6). Wax band was also seen to differ by 20%, 10% and 15% in NIA-0819, BL4, and NIA-98, respectively. It was also seen that the ivory markings were present on stalk of all the varieties and treatments. Moreover, width of the growth ring and the colour of the root ring also varied in all varieties. Occurrence of dwarf plant was very common at 30Gy and 40Gy while tall plants were observed in control, 10Gy, and 20Gy of the genotypes. Elahi *et al.* (2001) and Daniels *et al*. (1987) also reported significant variations in stalk colour, internode shape, bud groove, and bud colour of sugarcane caused by the gamma irradiation. Khan *et al.* (2009) and Samad *et al.* (2000), on the other hand, reported lethal effects of radiation on plant height. Sood *et al.* (2006) reported sugarcane variety CoJ 64, possessing enhanced cane height, cane yield and sugar yield as compared to parent, developed through similar approaches. In the present study, mutant variations generated among the plants can be used in breeding programme for the improvement of sugarcane cultivars.

The dendrogram resulting from cluster analysis of the sugarcane genotypes divided the accessions under study in to four major clusters (Fig. 1). It was evident from many of the observations of the cluster analysis that induced mutagenesis had caused significant variations among the genotypes, however parental background did have effect on such classification to some extent– especially in case of BL4 variety which formed its unique cluster against all other genotypes under study. Classification of accessions into different clusters is shown in Table 7, whereas means of the parameters for the clusters is presented in Table 8. Four major clusters were observed in the dendrogram developed on the basis of Euclidean distances among the genotypes tested. These clusters comprised of 15 genotypes, whereas two other genotypes namely NIA 98 (control|) and NIA 98 (20Gy) appeared distinguishing against the remaining genotypes forming separate clusters.

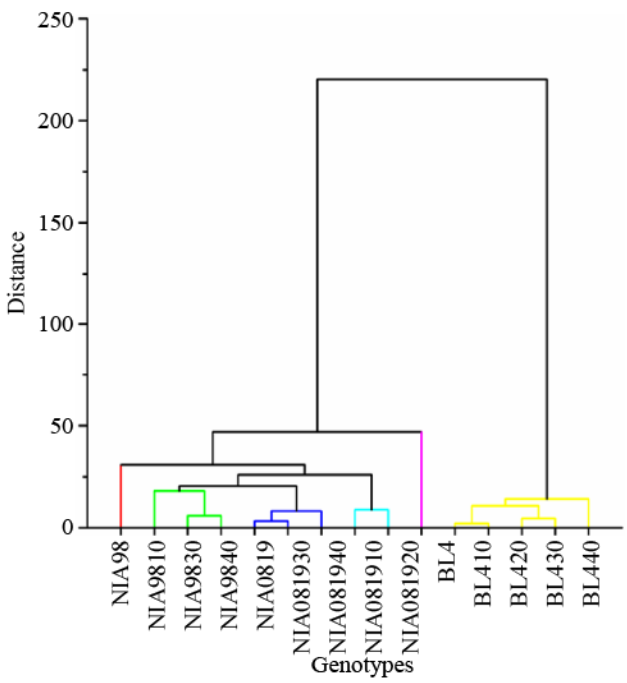

Fig. 1. Dendogram of the evaluated genotypes based on Euclidean distance.

Cluster I comprised of three accessions of NIA 98 (10, 30, and 40Gy). The cluster was distinguished by its highest sucrose%, sugar yield, and leaf width values. Cluster II, on the other hand, comprised of NIA0819 (control), and its 30 and 40Gy radiation treatments. This cluster was observed to be unique in terms of its highest leaf length, CCS% and purity%. Moreover, cluster III, embracing 10 and 20Gy treated genotypes of NIA0819 showed maximum quantitative traits (plant height, number of tillers/plant, stool weight, girth, number of internodes, internodes length and cane yield) coupled with low qualitative observations. Furthermore, cluster IV comprised of five accessions of BL4 (control, and 10, 20, 30 and 40Gy treated plants). This cluster was observed to have highest brix %, fiber %, and sucrose % values (Table 9).

Table1. Analysis of variance (mean squares) for various agronomic traits through induced mutations in sugarcane.

Source	DF	Plant height (cm)	Number of tillers plant^{-1}	Stool girth (cm)	Internodes length (cm)	Stool weight (kg)	Brix (%)	Fiber (%)	CCS (%)	Purity (%)	Sugar yield (tha^{-1})	Cane yield (tha^{-1})
Replications	2	451	0.4667	0.06067	3.8	0.5976	3.25163	12.1448	1.047	279.762	10.4227	59.76
Clones	2	445976**	94.8667**	0.01800ns	13844.9**	35.4042**	9.04793**	6.0055*	2.984*	279.762*	36.8892**	3540.42**
Treatments	4	1001**	4.5889**	0.10300*	11463.2**	4.9709**	2.99474*	0.6433ns	1.295ns	3.841ns	1.8955ns	497.09ns
C x T	8	320**	1.0056ns	0.04717ns	369.0ns	0.4967ns	3.1825ns	0.7679ns	2.378*	23.675ns	0.8251ns	49.67ns
Error	28	83	0.5619	0.02590	286.7	0.6268	25.5553	1.6545	0.959	44.637	1.5836	62.68

ns = Non-significant,* = Significant at 5% level, ** = Significant at 1% level

Table 2. Quantitative parameters of the genotypes.

Varieties	Gamma radiation doses (Gy)	Plant height (cm)	Number of tillerplant^{-1}	Stool weight (kg)	Stool girth (cm)	Number of internodes	Internodes length (cm)	Leaf length (cm)	Leaf width (cm)
	0	449 ab	9.66 bc	9.00 a-c	2.03 d	25.66 b-d	18.33 cd	133fg	4.40 bc
	10	441 bc	10.00 ab	9.63b	2.30 a-d	29.66 a	21.00 a	136f	4.16 b-d
NIA-98	20	441bc	9.00 ab	10.16a	2.46 a	27.33 a-c	20.00 ab	141e	5.20 a
	30	425 d	9.33 bc	8.66 bc	2.10 cd	29.00 ab	19.66 a-c	132 g	4.33 bc
	40	400 e	9.00 bc	9.13 a-c	2.10 cd	25.66 b-d	19.00 bc	122 h	3.96 c-e
	0	457a	8.66 c	9.00 a-c	2.16 b-d	24.33 c-e	17.00 de	173 b	4.07 b-d
	10	443 a-c	10.00 bc	9.50 ab	2.26 a-d	28.33 ab	21.00 a	174 b	4.14 b-d
NIA-0819	20	432.cd	11.00	10.00ab	2.36 a-c	28.66 ab	21.00 a	180 a	5.30 a
	30	428 cd	11.00	8.00 cd	2.13 b-d	26.00 b-d	19.36 a-c	162c	3.96 c-e
	40	429.cd	7.00 d	8.00 cd	2.36 a-c	24.00 c-e	18.70 bc	155d	3.53 ef
	0	137 f	5.33 e	6.66 e	2.03 d	20.33 f	14.00 gh	119hi	4.23 b-d
	10	140f	5.00 e	7.00 de	2.13 b-d	22.00 ef	16.00 ef	118 hi	4.26 bc
BL4	20	131f	6.00 de	7.33 de	2.30a-d	22.00 ef	15.00 fg	121 hi	4.50 b
	30	139 f	5.00 de	6.33 e	2.40 ab	22.00 ef	13.33 h	118i	3.80 de
	40	132 f	5.00 e	5.00 f	2.13 b-d	21.66 d-f	13.66 gh	117i	3.33 f
SE		7.44	0.61	0.64	0.13	1.70	0.80	2.06	0.21
LSD (5%)		15.24	1.25	1.32	0.27	3.49	1.64	4.23	0.44

Table 3. Qualitative characters of the genotypes.

Varieties	Gamma radiation doses (Gy)	Fiber (%)	Brix (%)	CCS (%)	Purity (%)	Sucrose (%)	Sugar yield (tha^{-1})	Cane yield (tha^{-1})
NIA-98	0	11.41 b-d	16.40 c	6.69 ab	68.09 ab	14.13 a	6.10b-d	90.00 a-c
	10	11.43 b-d	16.14 c	8.32a	75.78 a	11.41 b-d	8.42 a	100.00a
	20	12.70 ab	17.34 bc	7.85 ab	71.37 ab	11.43b-d	7.53 ab	96.33ab
	30	11.94 a-d	17.73 a-c	8.01 ab	70.92ab	12.70bc	7.28 ab	91.33 a-c
	40	12.00 a-d	18.60 ab	7.36 ab	66.84 ab	11.94b-d	6.34 a-c	86.67 bc
NIA-0819	0	10.84 d	16.58 c	7.45 ab	70.22 ab	12.00 b-d	6.73 a-c	90.00 a-c
	10	12.42 a-c	17.20 bc	6.89ab	65.91 ab	10.84 d	6.56a-c	95.00 ab
	20	11.39 b-d	17.07 bc	7.15ab	67.86 ab	12.42 bc	7.21ab	101.67 a
	30	11.04 cd	17.26 bc	7.64ab	69.72 ab	11.39 b-d	6.15b-d	80.00cd
	40	11.38 b-d	17.60ab	7.97ab	70.06 ab	11.04 cd	6.41a-c	80.00 cd
BL4	0	12.34 a-d	17.66 a-c	6.27ab	62.44 b	11.38 b-d	4.22ef	66.67 e
	10	12.46 a-c	18.20 ab	6.54ab	63.24 b	12.34 b-d	4.67c-e	70.00 de
	20	12.76 ab	18.60 ab	5.99b	60.72 b	12.46b-d	4.34 d-f	73.33 de
	30	12.54 a-c	19.06 a	6.45ab	61.54 b	12.76 ab	4.11ef	63.33 e
	40	13.18 a	18.98 a	7.02ab	63.92 b	12.54 a-c	3.63f	50.00 f
SE		0.768	0.7800	1.0503	5.4551	0.7998	1.0275	6.4645
LSD (5%)		1.57	1.597	2.1513	11.174	1.6383	2.1047	13.242

Table 4. Frequency of morphological changes in NIA-0819 variety as a result of induced mutagenesis.

Characters	Control	10 Gy	20 Gy	30Gy	40Gy	Characters mutants variant types %
Plant habit	Semi-Erect	Semi-Erect	Erect	Semi-Erect	Erect	35%
Tillering habit	Compact	Compact	Non-compact	Compact	Non compact	24%
Tillering density	Intermediate	Intermediate	Intermediate	Intermediate	**Profuse**	20%
Leaf colour sheath	Green lush	Green lush	Green lush	Green lush	Green	90%
Leaf length cm	133	136	141	132	122	
Number of green leaves	9	10	11	7	7	21%
Leaf lamin bland	4.40	4.16	5.20	4.33	3.96	
Width its broadest cm	Medium	Medium	High	Medium	Medium	
Outer auricle	Straight transitional	Straight transitional	Straight transitional	Straight and deltoid	Straight and deltoid	16%
Ligule	Crescent with broad lozenge	*	*	*	*	
Stalk colour	Yellowish green	Green	Yellowish green	Same	Yellowish	40%
Internodes shape	Obconoidal	Obconoidal	Cylindrical	Cylindrical	obconoidal	24%
Lover marking or cracks	Present	*	*	*	*	
Wax band	Heavy	Heavy	Heavy	Light	Heavy	10%
Bud	Obovate	Simple ovate	*	*	*	15%
Bud Size	Medium	Medium	Medium	Medium	Medium	
Bud Colour	Yellowish green /Brown	*	**	*	Yellowish green /Brown	10%
Bud Groove	Shallow	Shallow	Shallow	Shallow	Shallow	
Root primordia	Yellowish	Yellowish green and medium	*	*	Yellowish green and medium	
Root number of rows	2	2	2	3	2-3	6%
Flowering time	Nil	-	-	-		

Table 5. Frequency of morphological changes in BL4 variety as a result of induced mutagenesis.

Characters	Control	10 Gy	20 Gy	30Gy	40Gy	Characters mutants plant variant types%
Plant habit	Erect	Erect	Semi-Erect	Erect	Semi-Erect	30%
Tillering habit	Compact	Compact	Non-Compact	Non-compact	Non- compact	25%
Tillering density	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate and profuse	124%
Leaf blande	Light yellowish	Light yellowish	Light yellowish	Light yellowish	Green yellowish	80%
Leaf length cm	119	118	121	118	117	
Number of green leaves	9	10	12	11	8	33%
Leaf lamin bland	4.23	4.26	4.50	3.80	3.33	
Width its broadest cm	Medium	Medium	Medium	Narrow	Narrow	
Ligule	Crescent with broad lozenge	*	*	*	*	
Stalk colour	Yellowish green	Same	*	Light yellowish	*	20%
Internodes shape	Obconoidal	Cylindrical	Cylindrical	Cylindrical	Obconoidal	35%
Lover marking or cracks	Present	*	*	*	*	
Wax band	Heavy	Heavy	Heavy	Light	Heavy	10%
Bud Size	Medium	Medium	Medium	Medium	Medium	
Bud Colour	Yellowish green /Brown	*	**	*	Yellowish green /Brown	15%
Bud Groove	Shallow	Shallow	Shallow	Shallow	Shallow	
Root primordia	Yellowish green	Yellowish green and medium	*	*	Yellowish green and medium	
Root number of rows	2	2	2	2	2-3	5%
Flowering time	Nil	-	-	-		

Table 6. Frequency of morphological changes in BL4 variety as a result of induced mutagenesis.

Characters	Control	10 Gy	20 Gy	30Gy	40Gy	Characters mutants plant variant types%
Plant habit	Erect	Erect	Semi-Erect	Erect	Semi-Erect	22%
Tillering habit	Compact	Compact	Non-Compact	Non-compact	Non- compact	16%
Tillering density	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate and profuse	35%
Leaf blande	Light green	Light green	Light green	Light green	Green yellowish	95%
Leaf length cm	173	174	180	162	155	
Number of green leaves	9	10	10	7	6	20%
Leaf lamin bland	4.07	4.14	5.30	3.96	3.53	
Width its broadest cm	Medium	Medium	High	Narrow	Narrow	
Outer auricle	Straight transitional	Straight transitional	Straight transitional	Straight and deltoid	Straight and deltoid	
Ligule	Crescent with broad lozenge	*	*	*	*	
Stalk colour	Yellowish green	Same	*	Light yellowish	*	20%
Internodes shape	Obconoidal	Cylindrical	Cylindrical	Cylindrical	Obconoidal	35%
Wax band	Heavy	Heavy	Heavy and light	Light	Heavy	15%
Internodes habit	Obconoidal	Cylindrical	Cylindrical	Cylindrical	Obconoidal	25%
Bud	Obovate	Simple ovate	Narrow ovate	Simple ovate	Narrow ovate	30%
Bud Size	Small and medium	Medium	Small and medium	Small and medium	Medium	
Bud Colour	Yellowish green	*	**	*	Yellowish green /Brown	20%
Bud Groove	Shallow	Shallow	Shallow	Shallow	Shallow	
Root primordia	Colour and width	Yellowish green and medium	*	*	Yellowish green and medium	
Number of rows	2	2	4	2	2-3	7%
Flowering time	Nil	-	-	-		

Table 7. Classification of the genotypes in to various clusters.

Cluster	Accessions
Cluster I	NIA-98 10Gy, NIA-98 30Gy, NIA-98 40Gy
Cluster II	NIA-0819, NIA-0819 30GY, NIA-0819 40Gy
Cluster III	NIA-0819 10Gy, NIA-0819 20Gy
Cluster IV	Control BL4, BL4 10Gy, BL4 20Gy, BL430Gy, BL4 40Gy

Several studies have earlier explored the genetic diversity among sugarcane genotypes through similar approaches (Duarte *et al*., 2010; Dutra *et al.,* 2011; Sindhu *et al.,* 2011; Perera *et al.*, 2012; Santchurn *et al*., 2012; Santos *et al*., 2012). Sajjad & Khan (2009) hypothesized that the genotypes appearing from genetically similar parents does not contribute much towards the diversity among the progeny. Tahir *et al.* (2013) subjected sugarcane genotypes, evolved at different environmental locations, to cluster analysis and observed that the accessions appeared in groups on the basis of their geographical locations. Cluster analysis have been extensively employed for investigating the genetic similarity among different genotypes, as the genotypes appearing closer to each other in same clusters are expected to carry alike genetic make-up (Ghaderi *et al.,* 1980). We observed similar observations as most of the genotypes in our study classified on the basis of the parent they mutated from. The techniques have been employed in various other crops as well (Esmail *et al.,* 2008; Guerral *et al*., 2009). The selected mutants from these treatments may be further evaluated as new genotypes, and may also be utilized for further breeding progaramme as genetic stalk to improve the yield and qualitative traits in sugarcane. Hybridizing the genotypes appearing in different cluster is expected to play important role in getting diverse genetic combinations in the progeny plants.

Table 8. Characteristics of the clusters developed on the basis of Euclidean distance.

Parameters	Cluster I	Cluster II	Cluster III	Cluster IV
Plant height (cm)	231.536	428.665	432.339	136.194
Number of tiller plant^{-1}	6.844	8.165	8.699	5.266
Stool weight (kg)	7.286	8	8.7	6.464
Stool girth (cm)	2.187556	2.245	2.271	2.198
Number of internodes	23.70089	25	26.398	21.598
Internodes length (cm)	16.15422	19.03	19.49	14.398
Leaf length (cm)	122.6753	158.835	130.715	118.798
Leaf width (cm)	4.066	3.745	3.787	4.024
Fiber (%)	12.36733	11.21	11.488	12.656
Brix (%)	18.12116	17.4635	17.3327	18.5034
CCS (%)	6.919284	7.805	7.491	6.45726
Purity (%)	65.31162	69.8915	68.6889	62.3746
Sucrose (%)	12.20682	11.215	11.3824	12.3014
Sugar yield (t ha^{-1})	7.37	6.2845	6.5247	4.194
Cane yield (t ha^{-1})	73.99956	80	87.334	64.666

Table 9. Chanters selection from the clusters.

Cluster	Characters
Cluster I	Sucrose %, sugar yield, leaf width
Cluster II	Leaf length, CCS %, Purity %
Cluster III	Excellent quantitative traits (Plant height, number of tillers, stool weight, stool girth, number of internodes, internodes length, cane yield)
Cluster IV	Excellent qualitative traits (Brix %, fiber %, sucrose %, sugar yield)

Conclusions

The induced mutagenesis of sugarcane genotypes in the study resulted in significant differences among the mutants. 10Gy treated plants of NIA-0819were observed to have highest cane yield, whereas NIA-98 (10Gy) recorded the highest sugar yields. The gamma radiation doses of 30 and 40Gy showed negative effect on the cane yield in all varieties. Cluster analysis divided the genotypes into four major groups. Hybridizing the genotypes from different clusters can be of promising outcomes in terms of getting the genetic diversity among progeny plants for further evaluation in cane breeding programs.

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NIA-0819 20Gy

Figs. 2, 3, 4. Photograph of the sugarcane variety NIA-98, BL4 and NIA-0819 20Gy root zone, wax bands and bud shape.

Fig. 5. Photograph of the cane stalk of the sugarcane variety NIA-98 different doses of gamma irradiation showing the shape of internodes.

Fig. 6. Photograph of the cane stalk of the sugarcane variety NIA-0819 different doses of gamma irradiation showing the shape of internodes.

Fig. 7. Photograph of the cane stalk of the sugarcane variety BL4 different doses of gamma irradiation showing the shape of internodes.

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