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GAMMA RAYS INDUCED VARIABILITY FOR ECONOMIC TRAITS, QUALITY AND RED ROT RESISTANCE IN SUGARCANE (*SACCHARUM* SPP.)

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Abstract: Twelve hundred single bud setts of cv. Co J 64 were irradiated with gamma rays at 0, 10, 20, 25 and 30 Grays (Gy). The experimental material of M1V1 generation was planted during spring for its evaluation under field conditions. Data were recorded on ten economic and quality traits in addition to reaction to red rot disease against two pathotypes *viz.* CF 08 and CF 09. Analysis of variance revealed that mutagenic treatments generated significant differences for germination, number of tillers per clump, number of millable canes per clump, cane height, HR Brix, leaf length and single cane weight in M1V1 generation indicated the potential of mutagenic treatments for creating genetic variability for different traits in sugarcane. Average cane height among different treatments ranged from 166.60 to 185.80 cm with a mean of 171.2. Mean leaf length for different treatments varied from 106.30 to 114.80 cm with an average of 111.40 cm, maximum being 114.80 cm recorded for 10 Gy treatment followed by control. Cane weight an important trait in sugar cane ranged from 720.00 to 961.66 g. Maximum single cane weight was recorded for control followed by 10 Gy and 20 Gy treatments. The higher doses had a retarding effect on this trait. Artificial evaluation of irradiated subclones against two red rot pathotypes CF 08 and CF 09 using plug method revealed moderate resistance for all the mutagenic treatments. It is inferred that genetic variability could be induced in sugarcane using gamma rays for traits like germination, cane height, leaf length, HR Brix, single cane weight and disease tolerance. The lower doses of gamma ray (10 and 20 Gy) were more effective to induce variation while higher doses have detrimental effects. The sub clones need to be further evaluated in M1V2 for their consistency in trait manifestation and future use in breeding programme.

Keywords: *Saccharum*, Irradiation, Variability, red rot.

Introduction

Sugarcane is one of the important cash crops in India and plays pivotal role in both agricultural and industrial economy of the country. India is the largest producer of sugar, cultivating sugarcane in an area of 5.04 million hectares with a production of 338.168 million tones and productivity of 70 tonnes per hectare (Anonymous, 2014). However, the cane productivity and sugar recovery has not increased in last one decade in spite of expansion of

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the area. Several factors, like higher cost of cultivation, in-adequate irrigation facilities, breakdown of resistance of varieties to disease and insect-pests, natural calamities, climatic change etc are responsible for these phenomena (Ahloowalia, *et al.* 2004). Of these factors, diseases and insect-pests play a pivotal role. The loss in yield due to insect-pests and diseases has been estimated to the tune of about 20 per cent (Majid *et al*. 2001). Red rot epidemics were phenomenal in the past and responsible for considerable yield loss and for the elimination of many commercial varieties like Co 1148, CoJ 64 due to their break-down to red rot in India. The loss in cane yield and sugar recovery due to red rot varies from 0–100% depending upon the intensity of the disease. In a highly susceptible genotype, reduction in juice extraction (7.1–32.5%), pol % (7.4–38.7%), purity coefficient (0.5–8.3%), CCS (7.8–39.0%) and increase in reducing sugar (19.2–40.95%) have been reported (Khairwal *et al*. 1984). There is also deterioration in juice quality because sucrose contents decreases and the amount of reducing sugars, gums increase. These adversely affect the processing of white sugar in the mills. However, sustained breeding efforts led to deploying red rot resistant varieties in both tropical and subtropical regions and the disease was contained in the recent years. Successful use of plant breeding for improving crops requires the existence of genetic variation for useful traits but the desired variation is often lacking. Natural or induced genetic variability is a vital component of any plant breeding program. In sugarcane, diversity studies have shown that the modern commercial cultivars have been developed from a limited genetic base. Development of new sugarcane variety with stable performance has been a challenging task for sugarcane breeders. Induced mutations offer the possibility of generating desired attributes that either cannot be expressed in nature or have been lost during evolution (Brunner 1995). In sugarcane, most recessive mutants have been selected for the improvement of many characters. Several breeders have reported the successful use of mutations for developing genotypes resistant to diseases in sugarcane (Jagathesan *et al* 1974; and Srivastava et al 1986), sugarcane mosaic virus (Breanx 1975, Dermodjo 1977, Siddiqui and Javed 1982). Induced mutations in sugarcane have been reported for desirable traits and induce red rot resistant mutants of sugarcane variety Co S 687 using gamma rays and chemical mutagens (Siddiqui and Javed 1982). So, efforts have been made in present study to generate variability for yield and quality traits in addition to induce red rot resistance in sugarcane variety Co J 64 using gamma rays.

Materials and Methods

The experimental plant material used in the present study consisted of an important sugarcane variety Co J 64 developed from the cross (Co 976 x Co 617) having desirable attributes like early maturity, high sugar but susceptible to red rot. Despite extensive breeding efforts, till date no genotype has surpassed the early maturity and high quality characteristics of Co J 64, and it is still the quality check for the North West zone of the country. The variety was subjected to mutagenic treatment using gamma rays for generating variability.

Twelve hundred single bud setts of Co J 64 were subjected to water priming (primed and non-primed) prior to their irradiation. After that buds were taken to gamma chamber (Blood Irradiator 2000) for irradiation in the Department of Horticulture, Punjab Agricultural University, Ludhiana. Fifty buds were treated at a time in gamma chamber for different time intervals *viz*. 1 min 17 sec, 2 min 34 sec, 3 min 13 sec and 3 min 52 sec for 10, 20, 25 and 30 GY doses, respectively. The material generated from different treatments was used to grow first (M_1V_1) generation in the Sugarcane experimental area of Punjab Agricultural University, U.S.F, Ladhowal during spring 2012 for its evaluation under field condition. All the material was in planted in Complete Randomized Design having plot size of 10.5m ×2.25m with intra-row spacing of 30 cm comprising 34 buds per row. The plot size comprised of 10m x 3.65 m in complete randomized design. Recommended package of practices were followed to raise the healthy crop stand. The observations were recorded for germination (%) after 45 days and survival (%) after 120 days on plot basis; number of tillers and number of millable canes per clump and cane height (cm), cane thickness (cm), single cane weight (g) from 10 randomly selected canes; leaf length (cm) and leaf width (cm) from 5 leaves per treatment per repeat. Brixº was determined by using a hand refractometer (Erma, Japan) with scale ranging from 0 to 32˚ B for sugarcane juice (AOAC). The data recorded were analyzed according to completely randomized design analysis (Senedecor and Cochran 1967) using statistical software CPCS-1 package developed by Cheema and Singh (1990). The significance of variation among the treatments was compared by applying 'F' test and critical difference (CD) at 5 percent level of significance. The analysis of variance for each trait was based as per linear model suggested by Fisher (1954).

Evaluations of irradiated sub clones against red rot disease

The experimental material generated through different mutation treatments in present study was evaluated for red rot resistance. Red rot pathotypes CF 08 (from CoJ 84) and CF 09 (from CoS 767) were multiplied on oat meal agar medium in Petri dishes at 25±1ºC. For

inoculations, freshly sporulating 7-10 days old cultures were used. The spores were washed with sterile distilled water and homogenized by shaking and spore suspensions with concentration of $2X10^4$ conidia ml^{-1} was maintained. Experimental plant material was planted in the field area using single bud setts. Ten canes per treatment were inoculated by a suspension of two pathotypes viz. CF 08 and CF 09 by artificial inoculation under field conditions using plug method (Srinivasan and Bhatt, 1961). The inoculations were done in the third internode from the base of the standing canes injecting 1.0 ml of spore suspension ($2X10^4$ conidia ml^{-1}) with the help of hypodermic syringe. The core was then replaced and the openings were sealed with modeling clay. Disease data were recorded after 60 days of inoculation. The condition of the top was recorded and the canes splitted longitudinally. Observations were recorded on the number of internodes transgressed by the pathogen. The canes were rated 0-9 as per scale of Srinivasan and Bhatt (1961).

RESULTS AND DISCUSSION

Analysis of variance revealed non-significant differences among priming treatment for all the traits studied in M_1V_1 generation. However, mutation treatments revealed significant differences for germination, number of tiller per clump, number of millable caneper clump, cane height, HR brix, Leaf length and single cane weight while non significant for cane girth and leaf width of sugarcane in M_1V_1 sub clones of Co J 64 variety grown during spring 2012 (Table 1).

Table 1: Analysis of variance for different traits in sugarcane (M_1V_1) sub clones of Co J 64 during spring season 2012

Traits		**Mean Square due to**			
	Source of Variation	**Treatment**	**Priming**	**Treatment x Priming**	**Error**
	Degree of freedom	**4**	**1**	**4**	**20**
Germination (%)		330.19*	177.63	33.96*	4.33
Survival (%)		1600.63*	625.63	5.30	12.90
No. of Tiller per clump		7.10*	0.53	0.33	0.23
No. of Millable cane per clump		4.80*	0.53	0.33	0.40
Height (cm)		431.90*	52.50	1.30	12.70
Girth (cm)		0.79	0.31	0.20	0.15
HR brix (%)		2.20*	0.20	0.10	0.94
Leaf length (cm)		69.90*	0.97	0.41	9.60
Leaf width (cm)		0.62	0.75	0.28	0.67
Single Cane wt. (g)		38570.25*	79.56	356.65	395.75

* Significant difference at 0.05 level of significance

These results are in agreement with the findings of Kwon-Ndung and Ifenkwe (2000) who reported non-significant differences in the tiller population in subclones evaluated in M_1V_2 generation and significant differences for single stalk weight and HR brix. Earlier studies (Chaudhary 1971 and Premsekar and Appadurai 1981) also documented similar observation as reported in present study. The per cent germination of irradiated buds after 45 days of planting were recorded for different treatments revealed significant difference for this trait among mutation treatments and treatment x priming interactions. The germination percentage of irradiated buds ranged from 27.50 to 43.16 per cent in M_1V_1 generation grown during spring 2012 (Table 2). The highest germination was (43.16%) for control closely followed by mutation dose of 10 Gy. Germination percentage decreased with increase in gamma rays dose the lowest being for 30 Gy. Differential response of mutagenic treatment on single bud germination in sugarcane has also been reported by earlier workers (Kwon-Ndung and Ifenkwe 2000). The present results indicate that higher doses of gamma rays delay and suppress germination of sugarcane buds. These results are in complete agreement with the findings of Nwachukwu *et al* (1990). The number of plantlets survival after 120 days under field conditions showed that there were significant differences among treatments for per cent survival rate under both primed and non-primed conditions.

The highest per cent survival was recorded for control 0 Gy (86 %) and minimum for 30 Gy (47.66%). Of the different treatment combinations (TxP) interactions survival percentage ranged from 43.33% to 90.00% (Table 2). Effect of doses of gamma rays and ethyl methane sulphonate on germination and survival of induced mutations in pigeon pea has been documented by Premsekar and Appadurai (1981) who reported the reduction in survival with the increase in gamma dose. The mean number of tillers per clump ranged from 2.60 to 5.30 with an average of 3.80 when the buds were primed in water for 12 hrs where as it ranged from 2.30 to 5.00 when non primed fresh cut buds were sown after irradiation treatment. Among irradiation treatments highest number of tillers per clump was recorded in control (5.15) followed by 10 Gy (4.45) and minimum in 30 Gy (2.45). Yasmin *et al* (2011) who induced genetic variation in sugarcane through mutagenesis with doses of gamma rays 10, 20 30 and 40 Gy. They reported that different irradiation doses showed significant impact on average number of tillers, as the dose increased the tillering potential decreased. The mean number of millable canes per clump ranged from 2.30 to 4.00 with an average of 3.40 in the primed buds but it ranged from 2.30 to 4.00 in unprimed treatment with an average of 3.20.

Table 2: Performance of important characteristics of M_1V_1 irradiated sub-clones of Co J 64 grown during spring 2012

Traits	Priming	Mutagen Dose (Gy)					Mean	CD (5%)
		0	10	20	25	30		
Germination (%)	P	48.33	42.66	38.00	35.00	31.00	38.79	T=2.50, P=NS, TxP=3.23
	NP	45.00	39.00	34.00	29.00	25.00	34.4	
Mean		**46.66**	**40.83**	**36.00**	**32.00**	**27.50**		
Survival (%)	P	90.00	84.66	73.33	63.00	52.00	72.59	T=4.32, P=Ns, TxP=5.35
	NP	88.00	73.00	65.33	60.66	48.33	67.06	
Mean		**89.00**	**78.83**	**69.33**	**61.83**	**50.16**		
Tiller/clump	P	4.30	4.30	4.30	4.00	3.60	4.10	T=NS, P=NS, TxP=NS
	NP	4.60	4.30	4.30	4.00	3.60	4.16	
Mean		**4.45**	**4.30**	**4.30**	**4.00**	**3.60**		
Millable canes/clump	P	4.00	3.60	3.60	3.60	2.30	3.42	T=0.95 P=NS, TxP=3.54
	NP	4.00	3.60	3.30	3.00	2.30	3.24	
Mean		**4.00**	**3.60**	**3.45**	**3.30**	**2.30**		
Cane height (cm)	P	180.20	168.30	158.30	166.60	182.70	171.22	T=13.2, P=NS, TxP=4.75
	NP	183.30	178.50	157.40	177.20	185.80	176.44	
Mean		**181.75**	**173.40**	**157.85**	**171.90**	**184.25**		
Cane girth (cm)	P	2.10	2.30	2.30	2.30	2.30	2.26	T=NS, P=NS TxP=Ns
	NP	2.30	2.20	2.30	2.10	2.40	2.26	
Mean		**2.20**	**2.25**	**2.30**	**2.20**	**2.35**		
Leaf length (cm)	P	119.10	117.50	114.30	111.80	108.80	114.30	T=3.80, P=2.40, TxP=3.65
	NP	118.10	115.70	112.70	111.30	100.00	111.56	
Mean		**118.60**	**116.60**	**113.50**	**111.55**	**104.40**		
Leaf width (cm)	P	4.00	4.20	3.90	3.60	3.30	3.80	T=0.31, P=NS TxP=0.16
	NP	3.90	4.10	3.60	3.60	3.30	3.70	
Mean		**3.95**	**4.15**	**3.75**	**3.60**	**3.30**		
HR brix (%)	P	23.10	22.10	21.00	21.80	21.60	21.92	T=0.72 P=NS, TxP=Ns
	NP	22.30	22.00	20.90	21.90	21.50	21.72	
Mean		**22.70**	**22.05**	**20.95**	**21.85**	**21.55**		
Single cane weight (g)	P	975.66	981.66	876.66	795.00	706.66	867.12	T=28.75, P=1.70, TxP=4.54
	NP	961.66	971.60	826.66	780.66	710.00	850.11	
Mean		**968.66**	**976.63**	**851.66**	**787.83**	**708.33**		

NP- non priming, T- treatment, P-priming, T x P- treatment x priming

Mean number of millable canes was observed to be highest in control (4.00) followed by 10 Gy (3.60) and minimum in 30 Gy (2.30) having similar trend same as observed for number of tillers per clump. Various factors such as inhibition of auxin synthesis (Gurdon 1954), production of diffusible growth retarding substances (Mackey, 1951) and inhibition of DNA synthesis (Gaul 1970) have all been reported to be able to effect reduced growth in irradiated plants or seeds. Average cane height among different irradiation treatments ranged from 166.60 to 182.70 cm in water primed buds. The mean height was recorded to be 171.2. However, in unprimed treatment height ranged from 157.40 to 185.80 cm for different mutation treatments. Highest cane height of 185.80 cm was observed in 30 Gy. Khan et *al* (2009) have also reported higher cane height in gamma ray irradiated clones of sugarcane than control. They observed significant differences among treatment for this trait and recorded maximum plant height for gamma ray treatment of 20 Gy. They also found maximum cane height in clones of sugarcane variety NIA-2004 when mutagenic treatment was applied at the rate of 30 Gy. In present study, the dose of 30 Gy showed stimulating and enhancing effect on plat height which is in complete agreement with earlier findings of Khan *et al* (2007) who reported the genetic variability in sugarcane induced through mutation breeding. Refractometer brix in different mutation treatments used in present study ranged from 22.20 to 23.80 percent in primed buds with an average of 22.80 and which is at par with the values of brix in non primed buds. Maximum refractometer brix (23.80%) was recorded for 30 Gy treated population followed by control (23.40). Minimum refractometer brix (22.20) was recorded in population treated with gamma ray at the rate of 20 Gy. Similar to present results Khan et *al* (2009 and 2007) recorded low sucrose (%) and CCS (%) in 20 Gy and high in 40 Gy and 50 Gy, respectively.

Mean value of leaf length for different treatments varied from 106.30 to 114.40 cm with an average of 111.40 cm in primed and 105.70 to 114.80 cm with an average of 111.00 cm in non primed treatment. These results are in accordance with the findings of Hegde (2006) who studied effect of gamma irradiation dose on the vegetative characters in Turmeric (*Curcuma longa* L.) and reported that lower irradiation dosages have enhancing effect on leaf length. In this study, minimum dose i.e. 10Gy showed the maximum leaf length followed by the control. Cane weight is an important trait in sugar cane breeding programme. Due to low genetic variability in this crop exerted efforts are being made to enhance variability using different approaches. Single cane weight in irradiated sub clones of variety Co J64 ranged from 726.66 to 961.66 g in primed buds while the corresponding values for unprimed buds

were 720.00 to 931.66 g. Among various mutagenic treatments maximum single cane weight was recorded for control closely followed by 10 Gy and 20 Gy. The higher doses have retarding effect on this trait. Significant high primary stalk weight for lower gamma irradiation dose (20 Gy) as compare to 40 Gy has also been reported by Khan *et al* (2007) in sugarcane which is in complete agreement with present results.

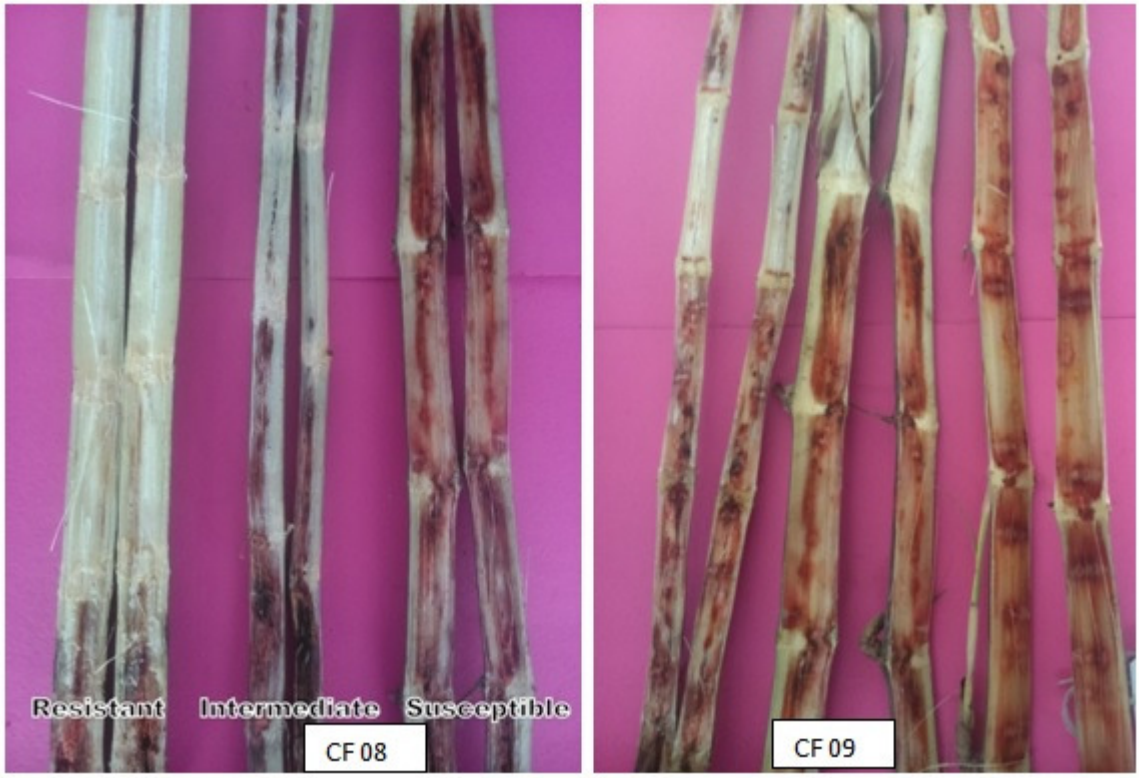
Table 3: Disease reaction of irradiated sub clones of CoJ 64 against two red rot pathotype in M1V1 generation

Gamma ray treatment (Gy)	Disease assessment score		Reaction	Per cent sub-clones
	CF 08	CF 09		
0	7.30	8.40	HS	100
10	3.20	3.30	MR	20
20	3.30	3.30	MR	20
25	3.40	4.60	MS	90
30	3.40	3.20	MR	10

MR- Moderately resistant, MS-Moderately susceptible

Perusal of Table 3 revealed that control population was highly susceptible to red rot disease. Reaction of irradiated sub-clones to two red rot pathotypes CF 08 and CF 09 under artificial inoculation by plug method revealed that among various mutagenic treatments the 10 and 20 Gy irradiated population, 20% canes obtained were found to be moderately resistant. But in 25 Gy irradiated population, 90% canes were moderately susceptible whereas in 30 Gy populations moderately resistant canes obtained were 10%. The moderately resistant and susceptibility reaction of clones are presented in Fig 1. The moderate resistance reaction among the mutation treatments, the disease score ranged from 3.20-3.40. These type reactions to red rot in mutated population of sugarcane has been reported by earlier workers (Ali *et al*. 2007, Majid *et al*. 2001)

From the present study, it is inferred that genetic variability could be induced in sugarcane using gamma rays for traits like germination, cane height, leaf length, HR brix, single cane weight and disease score. The lower doses of gamma ray (10 and 20 Gy) were more effective to induce variation while higher doses have detrimental effect on different traits especially germination and survival of sub-clones. The subclones identified for high single cane weight coupled with higher HR brix, cane girth and length and low disease score need to be further evaluated in M_1V_2 for their consistency in trait manifestation and future use in breeding programme.



Figs 1: Red rot reaction of irradiated subclones to CF 08 and CF 09 pathotypes by plug method in sugarcane

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