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In-vitro Mutagenic Studies in *Solanum viarum*

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ABSTRACT : Somaclonal variation for morphological traits was studied in both control and mutagen (chemical and physical) treated progenies of two varieties of *Solanum viarum*. The SC_1, SC_1M_1 (first generation following tissue culture) and SC_2, SC_2M_2 (second generation) of both 2n and 4n varieties on field evaluation showed bidirectional variation for plant height, plant spread, number of flowers and fruits per node and fresh fruit weight. Variation was more in number of fruits per node and fresh weight in physical mutagen treated progenies in both the generations. The qualitative variations observed were less spine plants, dwarf plants, sterile plant types and complete pollen sterility. The frequency of variations was different between varieties to varying degrees. SC_2 and SC_2M_2 progenies on evaluation revealed increased variability compared to seed control for all the traits indicating that these variabilities are not epigenetic in nature.

Keywords : In vitro mutagenesis, Somaclones, Seed control, *Solanum viarum.*

INTRODUCTION

Solanum viarum Dunal belongs to the family Solanaceae contains solasodine in its berries. The importance of this taxon as a source of solasodine was recognized as early as 1964, soon after the report on the high solasodine content in its berries by Maiti *et al.* (1964). The acceptance of this crop as an industrial source of solasodine, followed an acute shortage of diosgenin containing *Diascorea* tubers. Number of genetic amelioration programmes were conducted in this taxon during 1970's and 80's to get a variety with less spines and high solasodine content. Efforts were made to eliminate spines or mitigate this undesirable and irksome character and to select high berry yield with superior solasodine content through conventional breeding programmes and induced autotetraploidy (Ammal and Bhatt 1970; Bhatt 1975). Though, these genetic amelioration programmes were resulted in the development of several varieties, and objectives were not met completely. Perusal of literature revealed that induced in-vitro mutagenesis was not employed earlier in this taxon for genetic improvement. Hence, the present study is an attempt to obtain either spineless or less spine plants with high berry yield through in-vitro mutation breeding in diploid and tetraploid varieties of *Solanum viarum.*

MATERIAL AND METHODS

The seeds of diploid, Arka sanjeevini ($2n$ = 24) and tetraploid, Arka mahima ($2n$ = 48) varieties of *Solanum viarum* were procured from Indian Institute of Horticultural Research, Bangalore. Healthy plants were raised and maintained in the departmental garden, Botany Department, Bangalore University, Bangalore, India. Effective protocol for the regeneration of multiple shoots from the stem explants of both the varieties was reported previously (Tejavathi and Kumar 2003). For in-vitro mutagenic studies, stem explants of about 0.5 cm were exposed to chemical mutagen, Ethyl methane sulphonate- EMS and physical mutagen, Gamma irradiation at various concentrations and dosages respectively.

EMS Treatment

Surface sterilized stem explants of about 0.5 cm were dipped in aqueous solution of EMS at different concentrations ranging from 0.1 to 0.5% for 5 min.Morphogenetic potential of the culture was arrested above 0.5% of EMS. Treated explants were thoroughly washed in double-distilled water to remove the traces of mutagen and inoculated on Murashige & Skoog´s medium - MS supplemented with IAA (17.13µM) + BAP (8.87 µM) which was found to be the best combination and concentration of growth regulators for maximum multiple shoot regeneration (Tejavathi and Kumar 2003).

Gamma irradiation treatment

Source - ^{60}Co

Plant - KIRANA - Kidwai Institute of Oncology, Bangalore.

Dosage - for 2-6 min at 3 Kr.

Surface sterilized explants after inoculation on MS + IAA (17.13 µM) + BAP (8.87 µM) were brought to irradiation unit and exposed to different time intervals ranging from 2-6 min. After irradiation, the cultures were allowed to differentiate in the same medium. Longer period of dosage had resulted in arrest of morphogenesis.

Shoots were removed as and when they are formed in the cultures and transferred to rooting medium containing IBA at 4.90µM. After roots were formed, they were subjected to brief period of acclimatization by transferring them first to liquid ½ MS medium to sterile distilled water. Finally the plantlets were transferred to sand in pots and then to land.

Design of the experimental plot

As recommended by Reddy (1988) to get good yield responses, adaptation of square spacing in high planting densities in 30 × 30 cm for diploid and 45 × 45 cm for tetraploid varieties were selected. The berries were harvested at/after 180 days after field planting of treated/untreated tissue cultured plantlets

Nomenclature

SC_1 and SC_1M_1 have been used to denote the primary regenerates of tissue cultured and mutagen treated progenies which correspond to F_1 progenies respectively. SC_2 and SC_2M_2 have been used to term the first selfed progenies corresponding to F_2 progenies.

Statistical Interpretation of Data

Statistical interpretation of data was done by following Fishers analysis of variance technique (Pense and Sukhatme 1978). The results were composed at 1 and 5% level of significance. Mean values were compared by "*t*" test. The variants in morphological and yield characters were determined statistically as proposed by Fukui (1986). Finally SVF (Somaclonal Variation Frequency) was calculated by using the following formula.

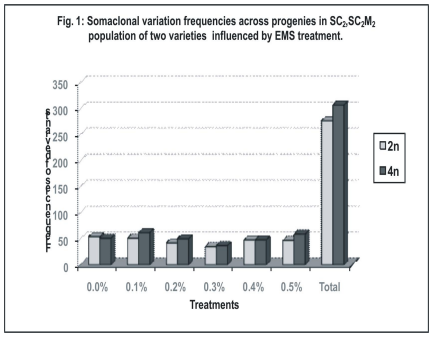
$$SVF = \frac{\text{Total number of variant plant in } SC_2 / SC_2M_2}{\text{Total number of plants in } SC_2 / SC_2M_2} \times 100$$

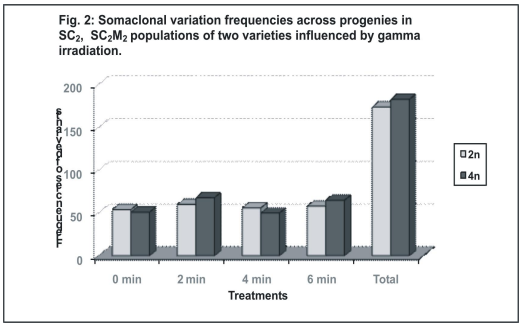
In the first generation SC_1 (untreated tissue cultured) was considered as control and SC_1M_1 (mutagen treated tissue cultured) as treated, whereas in second generation both SC_2 and SC_2M_2 progenies were served as treated (hormone and mutagen treated). The progeny raised from the seeds of the naturally grown parents was considered as control (ctrl). 12 plants in SC_1 and SC_1M_1 and 120 plants in SC_2 and SC_2M_2 generation were evaluated statistically for each treatment.

RESULTS AND DISCUSSION

Induction of mutation has been accepted as a useful tool in plant breeding programmes and it has contributed significantly to the wealth of genetic resources. One of the most important applications of mutation breeding is for the creation of variability in both qualitative and quantitative characters. The variability thus created enhances the scope for selection. However, in several plants, the culture in vitro itself has shown to be mutagenic especially when a callus phase was involved in the regeneration process (Larkin and Scowcroft, 1981). In the present investigation stem explants of two varieties of Solanum viarum were treated with EMS at different concentrations and exposed to gamma irradiation for varying periods to induce genetic variation. These treatments also affected the morphogenetic potential of the calli differently among the genotypes. However, in the varieties of Anthurium, genotypic specificity in regeneration capacity was not observed by Puchooa (2005) in the leaf culture.

Both chemical and physical mutagens affect the morphogenetic potential of the organogenic calli raised on MS + IAA (17.13 µm) + BAP (8.87 µm) which was established as a best hormone combination for multiple shoot regeneration (Tejavathi and Maruthi Kumar 2003). Number of shoots regenerated from the callus derived from 2n variety was more than that of 4*n* variety. However, the number of shoots regenerated in both the treatments of 2n and 4*n* variety is more than the control. The number and frequency of regeneration were however dependent on the concentration and dosage of the mutagen (Table 1 and 2). Gamma irradiation enhances the morphogenetic potential of the organogenic callus in both the varieties. Similar result was observed in wheat where 1.0 to 1.5 kr irradiation induces maximum plantlet regeneration from the embryo culture (He *et al.* 1993). However Sinha *et al.* (1996) observed inhibited regeneration in a few genotypes of sugarcane when they were irradiated. Regeneration frequency is also genotype dependent. Goud *et al.* (1969) reported that seedlings of purna, variety of finger millet were less regenerative compared to Annapurna and H22 varieties. The spectrum of variation unleashed by tissue culture and mutations are often different (Novak *et al.* 1988). However, variability can be created by combining tissue culture and low dosage of mutagen (Xiong and Zheng, 1989).





The regenerated plants of both control and treated were planted in the field with the recommended spacing of 30 × 30 cm for diploid and 45 × 45 cm for tetraploid after hardening. Observations were recorded for quantitative and qualitative variations in both first and second generations.

These observations are tabulated and presented in tables (Tables 3-8). Both quantitative and qualitative parameters were analysed in both EMS and gamma irradiated progenies and compared with untreated tissue cultured plants in first generation and with seed control in second generation.

Table 1 : Effect of EMS treatment at different concentrations on multiple shoot formation from stem cultures.

Var.	*Control*	*EMS treatment in different concentrations for 5 min*					*C*	*R*	*% R*
		0.1%	0.2%	0.3%	0.4%	0.5%			
2n	23.66±0.50	37.60±0.54	34.00±0.36	31.40±0.56	30.48±0.51	26.13±0.40	30	29.5	99%
4n	20.60±0.51	31.50±0.31	30.60±0.44	28.30±0.48	25.49±0.40	23.46±0.47	30	29.5	99

C-Control, R-Response

Table 2: Effect of Gamma irradiation at different time intervals on multiple shoot formation from stem culture.

Var.	*Control*	*Gamma irradiation*		*C*	*R*	*%R*
		2 min	4 min	6 min		
2n	23.66±0.50	39.66±0.54	35.00±0.44	31.60±0.61	30	29.5	99
4n	20.60±0.51	34.67±0.46	32.67±0.64	25.50±0.44	30	29.5	99

C-Control, R-Response

Table 3: Effect of EMS on quantitative characters in SC_1 and SC_1M_1 generations of diploid and tetraploid varieties of *Solanum viarum*.

Var.	*Conc.*	*Plant Height*			*Plant Spread*			*Flowers per node*			*Fruits per node*			*Fruit fresh weight*		
		CV	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*
2n	SC_1	25.31	107.16	15.96	32.31	106.41	1.69	29.07	3.87	1.10	29.03	3.85	1.08	58.35	6.30	6.05
	0.1	28.87	96.40	14.47	37.13	106.10	1.63	42.18	3.60	0.96	40.18	3.59	0.90	62.76	5.90	3.39
	0.2	29.22	93.84	13.47	37.72	105.70	1.52	44.40	3.90	1.37	42.39	3.87	1.34	60.41	6.02	5.10
	0.3	26.43	106.41	15.74	28.13	106.58	1.53	35.36	3.46	0.91	33.31	3.44	0.86	59.39	6.41	5.11
	0.4	24.30	108.18	15.42	28.46	105.80	1.46	48.20	4.38	1.60	46.24	4.36	1.54	57.33	7.03	6.44
	0.5	22.36	110.30	16.27	22.56	108.20	1.34	40.29	4.76	1.79	38.31	4.73	1.70	56.28	7.00	5.26
4n	SC_1	25.14	86.17	13.18	26.41	91.26	1.67	43.27	3.30	1.08	43.19	3.97	1.01	51.67	8.07	6.60
	0.1	23.11	88.31	13.84	26.30	91.10	1.61	50.00	3.69	1.26	48.20	3.65	1.24	45.43	10.09	12.67
	0.2	23.27	87.20	13.63	38.14	92.13	1.50	35.91	3.40	1.12	33.89	3.37	1.10	43.80	10.12	8.56
	0.3	22.46	101.34	15.10	26.19	91.16	1.51	43.18	3.48	1.18	42.30	3.46	1.16	48.71	10.00	8.00
	0.4	22.35	96.18	14.68	38.31	90.31	1.42	44.32	3.56	1.20	43.26	3.53	1.18	41.63	11.43	11.55
	0.5	21.31	94.30	14.47	38.21	91.30	1.31	43.97	3.20	1.11	41.50	3.17	1.14	49.50	10.02	7.56

CV- Co-efficient Variation, SD- Standard Deviation.

Table 4: Effect of Gamma irradiation on quantitative characters in SC_1 and SC_1M_1 generations of diploid and tetraploid varieties of *Solanum viarum*.

Var.	*Conc.*	*Plant Height*			*Plant Spread*			*Flowers per node*			*Fruits per node*			*Fruit fresh weight*		
		CV	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*	*CV*	*Mean*	*SD*
2n	SC_1	25.31	109.28	15.96	32.31	106.41	1.69	29.07	3.87	1.10	29.03	3.85	1.08	60.40	6.30	6.05
	2 min	29.76	108.31	11.30	28.41	108.30	1.60	31.28	3.91	1.56	30.16	3.89	1.54	62.46	6.48	3.58
	4 min	27.39	109.57	14.68	34.40	106.89	1.47	27.46	5.10	2.39	26.33	5.04	2.35	64.53	6.15	5.46
	6 min	21.48	109.57	17.21	28.21	108.49	1.43	23.50	5.79	2.30	20.47	5.74	2.27	58.70	7.16	5.30
4n	SC_1	25.14	95.27	13.18	26.41	91.26	1.67	43.27	3.30	1.08	43.19	3.25	1.01	57.38	8.07	6.60
	2min	11.34	94.40	14.31	26.58	91.58	1.59	33.18	4.37	2.79	31.16	4.34	2.74	39.33	12.12	12.10
	4 min	9.58	95.34	11.33	36.51	90.96	1.49	34.23	4.21	2.70	32.26	4.19	2.64	43.64	11.30	6.38
	6 min	25.74	95.20	13.48	26.71	91.87	1.41	34.30	4.10	2.73	33.27	4.09	2.70	47.70	11.10	7.91

CV- Co-efficient Variation, SD- Standard Deviation.

Table 5: Effect of EMS on quantitative characters in SC_2 and SC_2M_2 generations of diploid and tetraploid varieties of *Solanum viarum*.

Var.	Conc.	Plant Height				Plant Spread				Flowers per node				Fruits per node				Fruit fresh weight			
		PD	CV	Mean	t test	PD	CV	Mean	t test	PD	CV	Mean	t test	PD	CV	Mean	t test	PD	CV	Mean	t test
2n	Se. ctrl.	33.51	8.21	116.19	--	24.74	11.96	148.10	-	28.35	40.05	3.53	-	29.90	45.36	6.23	-				
	SC_2	56.99	14.35	114.87	1.32	28.88	13.00	138.60	5.80++	58.60	44.92	4.46	6.88++	48.39	65.97	6.26	0.15				
	0.1	48.73	13.00	112.16	4.29++	35.44	14.32	138.77	3.96++	53.80	50.37	4.51	6.50++	44.30	64.00	6.28	0.08				
	0.2	59.12	13.10	112.19	4.24++	33..33	13.64	138.63	5.94++	56.60	42.09	4.43	6.80++	55.97	59.31	7.96	3.01++				
	0.3	60.59	13.00	111.03	5.61++	53.53	19.31	138.31	8.94++	62.94	45.28	4.83	8.81++	46.47	66.29	5.66	1.1				
	0.4	48.21	13.13	111.66	4.68++	45.24	15.52	138.52	6.95++	54.17	53.89	4.22	4.67++	50.00	69.89	5.79	0.81				
	0.5	56.32	52.57	118.41	2.34+	47.70	14.35	138.36	9.40++	60.92	59.37	4.70	6.63++	59.20	65.28	6.88	1.49				
4n	Se. ctrl.	31.28	8.46	93.14	-	29.23	12.11	108.35	-	16.41	39.12	3.97	-	29.74	47.74	8.66	-				
	SC_2	70.56	10.97	78.84	3.56++	46.67	15.52	98.46	8.02++	36.11	43.93	4.77	5.43++	45.56	58.34	10.07	3.03++				
	0.1	37.23	7.94	85.22	8.62++	30.85	12.10	98.92	4.30++	40.96	43.29	4.85	6.03++	48.94	52.37	10.52	4.18++				
	0.2	40.88	9.48	92.07	1.03	40.33	12.80	98.77	5.60++	33.15	41.92	4.57	4.39++	54.70	57.10	9.90	2.73++				
	0.3	41.36	11.61	89.18	3.47++	35.60	12.12	98.72	6.39++	38.22	41.23	4.88	6.41++	48.69	61.20	10.02	2.85++				
	0.4	64.86	13.95	79.87	10.87++	40.00	14.44	98.55	7.50++	35.68	55.51	4.52	3.35++	48.11	67.50	7.98	1.57				
	0.5	42.37	8.08	86.49	7.03++	41.24	13.13	98.49	8.32++	41.24	46.39	5.11	7.03++	45.20	59.68	10.83	4.32++				

Se. ctrl = Seed Control, Duration of treatment = 5min, PD = Percent deviation, CV = Co-efficient Variation, ++, + = Significant at 1% and 5% respectively.

Table 6: Effect of Gamma irradiation on quantitative characters in SC_2 and SC_2M_2 generations of diploid and tetraploid varieties of *Solanum viarum*.

PD		CV	Mean	t test	PD	CV	Mean	t test	PD	CV	Mean	t test	PD	CV	Mean		t test
2n	Se. ctrl.	33.51	8.21	116.19	-	24.74	11.96	148.10	-	28.35	40.65	3.53	-	29.92	45.36	6.23	-
	SC_2	56.99	14.35	114.87	01.32	28.88	13.46	138.66	05.80++	58.60	44.92	4.46	06.88++	48.39	65.87	6.16	0.15
	2min	60.80	16.40	106.19	04.5++	40.75	14.36	138.10	06.00++	65.70	51.10	4.58	06.54++	50.71	59.66	7.69	2.90++
	4min	50.18	15.76	107.48	04.50++	66.16	16.10	137.90	07.61++	61.57	45.20	4.90	08.87++	64.07	66.40	06.9	2.40++
	6min	66.40	16.80	110.41	02.80++	48.40	14.73	138.40	09.48++	66.22	47.40	4.36	06.55++	62.90	57.68	7.95	3.00++
4n	Se. ctrl.	31.28	08.46	93.14	-	29.23	12.11	108.35	-	16.41	39.12	3.97	-	29.74	47.74	8.66	-
	SC_2	70.56	10.97	78.84	03.56++	46.67	15.52	98.46	08.02++	36.11	43.93	4.77	05.43++	45.56	58.34	10.07	3.03++
	2min	44.90	10.30	19.01	01.01	45.30	12.90	97.68	05.50++	44.90	43.20	4.86	06.04++	56.55	60.30	10.04	2.90++
	4min	70.86	14.80	74.81	11.03++	40.09	14.66	96.40	07.60++	35.61	55.47	4.70	03.31++	50.52	69.99	10.87	4.36++
	6min	44.71	09.14	83.40	08.01++	58.02	14.33	96.31	08.40++	46.52	49.40	5.14	07.00++	45.12	59.80	10.60	4.30++

Se. ctrl. = Seed control, Duration of treatment = 5min, PD = Percent deviation, CV = Co-efficient Variation, ++, + = Significant at 1% and 5% respectively.

Table 7: Proportion of progenies exhibiting qualitative variations in SC_1, SC_1M_1, SC_2 and SC_2M_2 generations of diploid and tetraploid varieties of *Solanum viarum* in EMS treatment.

Var.	*Character*	*Tissue cultured*		*0.1%*		*0.2%*		*0.3%*		*0.4%*		*0.5%*	
		SC_1	*SC_2*	*SC_1M_1*	*SC_2M_2*	*SC_1M_1*	*SC_2M_2*	*SC_1M_1*	*SC_2M_1*	*SC_1M_1*	*SC_2M_2*
SC_1M_1	*SC_2M_2*										
2n	Dwarf	0(0.0)	2(19.6)	0(0.0)	15(13.6)	0(0.0)	14(12.7)	1(9.0)	14(12.0)	0(0.0)	20(18.9)
0(0.0)	21(19.2)										
	Pollen sterility	0(0.0)	0(0.0)	1(9.0)	0(0.0)	2(18.0)	5(3.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
0(0.0)	6(4.3)										
	Sterile plant	1(9.0)	4(2.2)	2(18.0)	1(0.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)
1(9.0)	0(0.0)										
4n	Dwarf	1(9.0)	22(20.0)	1(9.0)	0(0.0)	2(18.0)	5(3.0)	1(9.0)	0(0.0)	1(9.0)	0(0.0)
1(9.0)	6(4.3)										
	Pollen sterility	1(9.0)	17(15.2)	3(27.0)	23(21.1)	0(0.0)	19(16.4)	0(0.0)	18(16.0)	1(9.0)	26(24.0)
0(0.0)	19(17.0)										
	Sterile plant	0(0.0)	0(0.0)	1(9.0)	00(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(9.0)	0(0.0)
0(0.0)	3(1.4)										

Figures in the parenthesis indicate percentage.

Table 8: Proportion of progenies exhibiting qualitative variations in SC_1, SC_2, SC_1M_1 and SC_2M_2 generations of diploid and tetraploid varieties of *Solanum viarum* in gamma irradiation treatment.

Var.	*Character*	*Tissue cultured*		*2 min*		*4 min*		*6 min*	
		SC_1	*SC_2*	*SC_1M_1*	*SC_2M_2*	*SC_1M_1*	*SC_2M_2*	*SC_1M_1*	*SC_2M_2*
2n	Dwarf	0(0.0)	21(19.6)	0(0.00)	17(15.3)	0(0.00)	10(8.2)	0(0.00)	22(20.2)
	Pollen sterility	0(0.0)	0(0.0)	0(0.00)	0(0.00)	1(9.00)	3(1.0)	0(0.00)	5(30)
	Sterile Plant	1(9.0)	4(2.2)	3(27.00)	1(0.8)	0(0.00)	1(0.8)	0(0.00)	0(00)
4n	Dwarf	1(0.0)	22(20.0)	3(27.00)	14(12.0)	1(9.00)	5(30)	0(0.00)	0(0.00)
	Pollen sterility	1(9.0)	17(15.2)	0(0.00)	10(8.0)	3(27.0)	6(4.0)	1(9.00)	12(10.0)
	Sterile Plant	0(0.0)	0(0.0)	0(0.00)	0(0.0)	1(9.00)	0(0.0)	0(0.00)	0(0.0)

Figures in the parenthesis indicate percentage.

The study of second generation of the regenerated plants would help to differentiate between heritable and non-heritable variations, express recessive mutations and do away with the in vitro stress-induced variations observed in the first generation. Cheng *et al.* (1992) found that somaclones in SC_1 were highly variable mainly due to physiological disturbances resulting from the in vitro culture. Some of the unwanted alterations induced by in vitro culture are partly eliminated by subsequent cycles of sexual reproduction and can be reduced further by selections of individual plants showing desirable variations.

In vivo evaluation and comparison

A. SC_1 and SC_1M_1 generation

Quantitative variations : Agronomically important quantitative characters such as plant height, plant spread, number of flowers, berries per node and fresh weight of berries were studied. The plant height and spread were maximum when plant reaches 180 days of age in both the varieties. Hence data was taken after 180 days of growth. There was new variability contributed to plant height in terms of wider range, shift in mean and higher CV for plant height in SC_1 generation. Similar observations were made in several crop species including rice (Jun *et al.*, 1986), wheat (Ahloowalia and Sherington, 1985) and maize (Zehr *et al.* 1987). In SC_1M_1 generation of 2*n* variety, tallest plant was observed in 6 min irradiation treatment which was taller than the tallest of EMS treatments and control. Whereas shortest was observed in 2 min irradiation treatment which was shorter than the shortest of EMS treatment and control. While in 4n derived SC_1M_1 generation, tallest was recorded in 0.3% EMS treatment which was taller than the tallest of gamma irradiation and control. Shortest was observed in 4 min irradiation treatment which was shorter than shortest of EMS treatment and control. In general, 4n derived SC_1M_1 generation showed a shift in the plant height to lower side due to irradiation treatments and higher side due to EMS treatment. Co-efficient of variation varied unusually with the highest value of 29.76 in 2*n* derived progenies at 2 min irradiation treatment and lowest value of 9.58 at 4 min irradiation treatment in 4n derived SC1M1 progenies. Reduction in plant height of somaclones has been reported in rice (Fan *et al.* 1991). While Adkins (1993) noticed increase in plant height in SC_1 plants of rice.

Plant spread of two varieties of SC_1M_1 generation remained more or less similar to that of control (SC_1) in both the treatment. However, the co-efficient of variation ranges from 22.56 in 2*n* derived progenies at 0.5% EMS treatment to 38.31 in 4*n* derived progeny at 0.4% EMS treatments. Kumarswamy and Krishnan (1988) studied the morphological characters of the less spiny Glaxo variety of Solanum viarum with four diploid less spiny entries developed at IIHR, Bangalore and 14 C_5 generation induced autotetraploids of Glaxo variety. Tetraploids recorded lower plant spread in both the directions compared to diploids. Ravindra *et al.* (2004) however reported wider plant spread in SC_1 generation of rose scented geraniums. They attributed these varieties to physiological causes and interplant spacing.

The data for number of flowers per node was recorded during 90-95 days after their planting for 2*n* variety and 125-130 days for 4*n* variety, since the number of flowers was found to be maximum during these days. Highest mean for number of flowers per node (5-7) was recorded at 6 min irradiated 2*n* variety progenies which was more than the control and EMS treatment. Whereas lowest mean of 3.2 was obtained in 0.5% EMS treated 4*n* variety progenies. However CV values were lower in most irradiation treatments compared to CV values of EMS treated progenies. Increase in the number of flowers per plant in SC_1 somaclones of smooth brome grass has been documented (Wattanasiri & Walton, 1995).

Number of fruits per node was recorded after 180 days, because whatever yield available at these days were suitable for harvest. Therefore yield available for harvest was considered as actual yield. The highest mean of 5.74 was found in 6 min irradiated 2n variety derived progenies whereas the lowest mean of 3.17 was recorded in 0.5% EMS treated 4*n* variety. Nevertheless CV values in irradiated derived progenies were lower in most of the treatments compared to CV values of EMS treated derived progenies.

The variation for fresh weight of fruits was more as a result of tissue culture and in vitro mutation in both the varieties. In 2*n* variety, range increased with the irradiation dose and EMS concentration. In both the varieties, SC_1M_1 generation recorded the higher fresh weight than SC_1. The highest mean of 12.12 was recorded in 4n variety progenies at 2 min irradiation treatment. Whereas the lowest mean of 5.90 was obtained in 0.1% EMS treated 2n variety. The CV values were generally high for fresh weight of berries in both the varieties. Based on these results, it can be concluded that the tissue culture, along with mutagen treatments can generate more variation in yield as a compounded effect of induced variations in yield attributes. Bhatt and Heble (1978) reported an increase in the average weight of fruits/plants in irradiated diploid mutants of *Solanum khasianum*.

Qualitative variations : Variations in qualitative traits in mutation and tissue culture have been widely reported (Earle and Gracen, 1985; Sun *et al.* 1993). These variations may or may not be of agronomic importance, although they add to our understanding of the genetics of linked traits and different metabolic systems. The spectrum of variation observed in SC_1 and SC_1M_1 generations included less spiny/ spineless plants, sterile plant types, dwarf plant types and complete pollen sterility. Occurrence of spineless plants were common irrespective of all the treatments in SC_1 and SC_1M_1 generation. Khanna *et al.* (1976) observed the same in regenerated plants of *S. khasianum*. If at all one or two spines are present on the dorsal surface of the leaves they are blunt or curved. Dwarf plant types were higher in gamma irradiated progenies of 4*n* variety. Maximum of 27% was recorded in 4*n* variety irradiated for 2 min, whereas 4n

variety control (SC_1) showed only 9%. While 2n variety exhibited the same frequency at 0.3% EMS treatment. Dwarf variants were not noted in 2*n* control (SC_1) progenies. Sun *et al*. (1993) have reported dwarf mutants in rice somaclones. The frequency of variation was however less in the non-irradiated SC_1 compared to SC_1M_1.

Maximum percent of sterile plant types (27%) were recorded in 2*n* variety at 2 min irradiation treatment. Comparatively maximum percent of sterile plant type variants were recorded in gamma irradiated treatments than EMS treatments. There are several reports of sterile plant types of non flowering plants in SC_1 generation of many crops like wheat (Eapen *et al.* 1985) and barley (Ahloowalia 1987). However, Wattanasiri and Walton (1993) did not encounter sterile plant types in smooth bromegrass cultures. Highest percent of complete pollen sterility variants (27%) was recorded in 4*n* variety at 0.1 EMS treatment and 4 min irradiation treatments. In regular mutation studies seed irradiation brings pollen sterility of different degrees in crop plants. According to Nandakumar (1983) autotetraploids could be easily recognized from diploids based on their low pollen fertility. Sreekantaradhya (1979) observed reduction in fertility upto 80.20% in 50 kr of gamma radiation seed treatment in *Eleusine coracana*. Decrease in pollen fertility in SC_1 generation of rice (Fan *et al.*, 1991) and barley (Ahloowalia 1987) have also been documented.

B. SC_2 and SC_2M_2 generation

SC_2 and SC_2M_2 progenies were obtained from the seeds of SC_1 and SC_1M_1 progenies respectively. Their growth performance was compared with seed control progenies, which were raised from the seeds of naturally grown parents and served as control. The morphological parameters that were analyzed for first generation were studied even in this second generation.

Quantitative variations : In both SC_2 and SC_2M_2 progenies plant height generally lowered below that of seed control in both the varieties. It was also observed that there were more plants with lower height as compared to control in all treatment of both varieties. Higher frequencies of deviants (70.86%) were recorded in 4*n* variety of 4 min irradiation treatment. CV generally increased above that of control in both the varieties at all treatments which rather a reflection of frequency of variants. Progenies of both the treated varieties varied significantly from the seed control for mean and variances as confirmed by't' test. Reduction in plant height was reported in somaclones of Paspalum species (Davies and Cohen 1992) Rice (Fan *et al.* 1991), wheat (Kardimora 1993) and maize (Dolgikh *et al.* 1992). Nayar *et al.* (1974) have also reported plants with lower height mutants in M_2 generation of finger millet varieties.

Lower mean values for plant spread were recorded in all the treated progenies compared to seed control in both the varieties. Though the frequency of deviants increased in relation to seed control in all the treatments, the change was mostly towards negative side. It is the reflection of increased incidence of plants with lower plant spread as expected, the't' test indicated that these treatments to be marginally different from the control in both the varieties. Reddy (1988) evaluated 22 diploid and 18 C_6 generation induced autotetraploids lines of *Solanum viarum* for their morphological characters. He observed parity for plant spread in both directions. Ravindra *et al.* (2004) however reported wider plant spread in SC_1 generation of rose scented geraniums and decreased spread in SC_2 generation.

While a modest range of 1 to 6 (2*n*) or 1 to 7 (4*n*) flowers per node were observed in the seed control, maximum number of 14 (4*n*) and 22 (2*n*) flowers per node in EMS treatment and 14 (4*n*) and 18 (2*n*) flowers per node in irradiated treatments were recorded. Most of the deviations in these treatments were falling in the high flower number range in both the varieties. Significant increase in mean was noticed between the seed control and the treatments in both the varieties. All the treatments were differed significantly from the seed control in both the varieties as indicated by 't' test. The early flower falling was successfully checked for crucial five days in both the varieties that is between 90-95 days for 2*n* variety and 125 to 130 days for 4n variety by good agro management practices such as in time water feeding, manure applications, pest control and favourable environmental conditions. Hence, the above mentioned statistical analysis for flowers/node holds good for fruits/node parameter also in all the treatments for both the varieties. Ammal and Bhatt (1971) in *Solanum khasiamum*, Meheswar (1983) in *S. viarum* var. BARC and Gowda *et al.* (1987) in *S. viarum* also reported higher number of fruits per node in C_2 generation of autotetraploids. According to Bhama (1991) both in diploids and tetraploids of *Solanum viarum*, in addition to berry number, contribution of big sized berries to berry yield proved very important as these characters have selection value. However, Mahadevu (1998) had observed negative shift in mean in *Vigna* spp.

The variation for fresh weight of fruits was more as a result of in vitro culture and in vitro mutagenesis in both the varieties. The fresh weight per berry of SC_2 and SC_2M_2 generation increased over seed control in all the treatments of both the varieties. In all the treatments widening of the range was observed. Highest mean of 10.87 was recorded in 4*n* variety at 4 min irradiated treatment which is higher than the seed control (8.66). CV was very high than that of seed control in all the treatments of both the varieties. The increased in range was reflected by the frequency of deviants, CV and mean values in gamma irradiated progenies. The increase in variability in both the directions was a clear indication of polygenic variability released by tissue culture and in *vitro* mutagenesis.

Qualitative variations : Less spine/spineless character was retained in SC_2 and SC_2M_2 generation indicating its hereditable nature. Rev and Bras (2003) isolated spineless somaclones from the cultures of pineapple. Dwarf plant types were observed in 2*n* variety among all EMS treatment. Comparatively less dwarf plants were observed in 4*n* variety

in both the treatments compared to 2*n* variety in SC_2 and SC_2M_2 generation though highest number of 22 plants (20%) was observed in SC_2 progeny of 4*n* variety. Gamma irradiation treatments promoted the dwarfness in both 2*n* and 4*n* varieties except at 6 min irradiation treatment in 4*n* variety. However, dwarf plants were more in SC_2 generation. Sun *et al.* (1993) reported dwarf mutants in rice somaclones. Complete pollen sterility variants were observed in the progenies of 4n variety in all the treatments of EMS and gamma irradiation. 4n variety recorded maximum of 12 variants at 6 min irradiation and 26 variants at 0.4% EMS treatments. Decreased fertility in SC_2 generation was recorded in rice (Oono *et al.* 1986) wheat (Chen *et al.* 1987) and sorghum (Ma *et al.* 1986).

Sterile plant types or non-flowering plants were negligible percent in both the varieties. They are completely absent in 4*n* variety except in 0.5% EMS treatment. However, sterile plant types were observed among the progenies of Basmathi rice somalcones (Abbas *et al.* 1988).

Somaclonal variation vs. in vitro mutagenesis

Frequency of variant progenies in SC_2M_2 was more than SC_2 for many characters both quantitative and qualitative. Xiong and Zheng (1982) suggested that issue culture coupled with in vitro mutagenesis increases the frequency of variations. It is the indication of mutagenesis affecting the proportion of somaclonal variants. Abeysekera (1992) suggested that tissue culture accompanied with mutagen treatment is highly effective. Another important factor noted was that SC_1 plants had lesser progenies of variants than SC_1M_1 and was continued to SC_2 and SC_2M_2 generation.

***Somaclonal variation frequency* (*SVF*)**

It refers to the proportion of detectable variants in a given population. SVF is significant in both the treatments. While SC_2 generation recorded the SVF of 45.10% for 2n and 43.84% for 4n, EMS treatments recorded the SVF of 46.51% for 4*n* and 42.31% for 2*n*. Whereas in gamma irradiation, the maximum of SVF of 50.39% for 2n and 53.46% for 4*n* were recorded. On an average, 4n had high SVF compared to 2*n* variety. The comparable SVF observed among treatments suggest in vitro mutagenesis may be of an extra significance compared to in vitro culture in term of frequency and spectrum of variants generated (Figs. 1 and 2).

CONCLUSION

The most significant aspect of the present study has been the demonstration of generation of new variability for economic traits like fruit yield contributing characters. The presence of such polygenic variability can only be revealed by using simple basic statistic parameters like mean, range and variance. The generation of this new genetic variability should permit gain by selection for more desirable levels of such traits. Other significant phenotypic variation that was observed in all the regenerated plants is the absence of spines all over the body. Since this variability is agriculturally important and therefore, easier to manage in the field during the harvesting period, coupled with high yielding traits, is a significant breakthrough in the breeding programmes of this taxon.

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