

Induced Genetic Variability for Yield and Yield Traits in Basmati Rice

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Abstract: The mutagenic effect of gamma rays, ethyl methane sulphonate (EMS) and sodium Azide (SA) used either singly or in combination were observed for induced genetic variability for yield and yield traits in M₂ and M₃ generation in the two cultivars of Basmati rice. The observation mean range and coefficient of variation (CV) suggest that the mutagen treatment had wider values than the control. The majority of the mutagenic treatment induced negative shift for the mean away from the control. The observed changes in both the generations revealed that the magnitude of variability declined from M₂ to M₃ generation. The mean performance showed improvement in most of the mutagenic treatments in M₃ as compared to the corresponding treatments in M₂ generation. The magnitude of CV in the treated populations as generally higher than the control for most of the traits in both the generations for both the varieties. All the three mutagens were quite effective in inducing genetic variability in Basmati rice. However, 1.5 kR, 25 kR and 30 kR dose of gamma rays, 0.03 M EMS, 1.5 mM SA, 1.5 kR gamma rays + 0.03 M EMS, 20 kR gamma rays + 0.0 M EMS, 10 kR gamma rays + 1.5 mM SA and 20 kR gamma rays + 1.5 mM SA were found more effective in inducing genetic variability.

Key words: Gamma rays % EMS % SA % mutagenic and yield

INTRODUCTION

The primary objective of induced mutation is to enlarge the frequency and spectrum of mutations and also increase the incidence of viable mutations as an approach towards directed mutagenesis [1]. With this view, the present investigation was undertaken to study the mutagenic effects of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (SA) employed individually or in combination for six quantitative traits in M₂ and M₃ generations in Basmati rice [2]. Induced mutation is an important tool in rice breeding worldwide [3,4]. To increase the yield potential, it is necessary the assembly of many techniques that form modern genetic and breeding program such as empiric selection, MAS and genomic methods [5]. Also changes in the paradigm of phenotypic selection and disregarding differences between classical and modern breeding as well as between transgenic and non-transgenic plants have been suggested [6]. Mutation techniques are very important tools to study the genetic variability, function, action and regulation of genes, moreover in plant breeding. In this paper we propose the use of mutation technique to

directly select mutants affecting yield traits of Basmati rice. Ionizing radiations have been successful including genetic variability in rice. Before the start of any sound breeding program knowledge of relative biological effectiveness and efficiency of various mutagens is useful in mutation breeding [7]. A number of attempts have been made by different group of workers in the direction to determine the most effective mutagenic treatments for the induction of desirable and marketable traits in rice [8-15]. Basmati rice is premier food grain crop of poaceae family. Basmati rice is grown in Asian countries for domestic consumption and export also. Genomics has contributed to comparative studies especially after the recent development of molecular marker technology and bioinformatics [16-19]. The main objectives was to estimate mutagen effects on yield component of rice.

MATERIALS AND METHODS

The dry seeds (contains 12 percent moisture,) of the two varieties of Basmati rice, namely, Taraori Basmati and Pusa Basmati 1 were exposed to mutagenic treatments [20]. Two hundred seeds were taken for each mutagenic

treatment from both the varieties. The seeds were sealed in polythene bags and irradiated to 5 kR, 10 k, 15 kR, 20 kR and 30 kR doses of gamma rays (^{60}Co source) of Gamma garden at IAR New Delhi [20]. Various concentrations of two chemical mutagens, viz, ethyl methane sulphonate (EMS) Sodium azide (SA) procured from Sigma Chemical Company, USA were employed in the present investigation [21,22]. The solutions of EMS and SA were prepared in phosphate buffer at pH7.0 and 3.0 respectively. The various concentration of EMS (0.02M, 0.03M, 0.04M and SA 0.5mM, SA 1.0mM, SA 1.5mM, SA 2.0mM) was used. For chemical treatments, the seeds were soaked in distilled water for six hour to ensure complete hydration of the seeds. There after, the seeds were treated with solution of EMS or SA for a period of six hours in the laboratory conditions [23].

The seeds were intermittent shaking throughout the period of treatment to maintain uniformity. After treatment, the seeds were thoroughly washed in running water for one hour to remove traces of residual chemicals [24].

The combination treatments of gamma ray and EMS were as follows 10kR +0.03M, 15kR + 0.03M, 20kR+0.03M, 10kR +0.04M, 15kR +0.04M and 20 kR + 0.04 M. The combination treatment of gamma rays and SA were as 10 kR + 1.0 mM, 15 kR + 1.0 mM, 20 kR + 1.4mM, 10 kR + 1.5 mM, 15 kR + 1.5 mM and 20 kR + 1.5 mM. In addition, a combination treatment of gamma rays (5kR EMS (0.02M) and SA (0.5 mM) comprising 5 kR, 0.02 M and 0.5 mM was also administered. In the combination treatment, the seeds of both the varieties were first exposed to gamma rays at 10kR, 15 kR and 20 kR doses and then soaked in the respective concentrations of EMS or SA as mentioned above [25].

The field experiment was conducted at the Research Farm, Institute of Agricultural Science, BHU, Varanasi. The M1 generation was grown during kharif, 2005 The M2 was raised in kharif, 2006 while the M3 generation was grown in kharif, 2007 in RBD with three replications./The recommended cultural practices were followed during the crop growth period. The observations regarding micro mutational studies in the M2 and M3 generation were recorded on days to 50% flowering, plant height, panicle length, number of panicle bearing tillers per plant, 100-seed weight and grain yield per plant [26]. The data for these traits were recorded on 50 normal looking plants from each treatment and also from the control from each replication of both the varieties in M2 and M3 generations.

RESULTS AND DISCUSSION

The data on induced variability as reflected by the range, mean and coefficient of variation (CV) in Taraori Basmati 1 and Pusa Basmati are presented in Tables 1-4.

Days to 50% flowering: It is evident from the results that in Taraori Basmati, the shift towards late flowering was observed maximum at 15 kR gamma rays + 1.0 mM SA in M2 and 10 kR gamma rays + 0.03 M EMS in M3 generations. The values of CV increased in all the treatments as compared to the control in both the generations. The extent of variability was higher in M2 than M3 generation in both the varieties in both the generations. In case of Pusa Basmati 1, the shift towards late flowering was observed maximally at 10 kR gamma rays + 0.04 M EMS in M and at 15 kR gamma rays in M3 generations. In general, there was a wider range for days to 50% flowering in the treated population than the control.

Plant Height: It is obvious from the observations (Table 1) that there was shift in the mean in positive as well as negative directions over the control. Similarly, there was wider range in the treated population as compared to the untreated population. The extent of variability was higher in M2 than M3 generation. Invariably, the variability increased in both the generations, irrespective of increase or decrease in the mean values. Plant height increased or decreased in the mean values. Plant height increased or decreased significantly in both the varieties in both the generations. The shift towards increased plant height was maximum in M2 and M3 generations at 15 kR gamma rays + 0.04 M EMS in both the varieties.

Panicle Length: It was apparent from the results (Table 2) that there was shift in the mean towards positive and negative direction over the control in both the varieties in both the generations. The CV for panicle length was invariably higher in the treated population, irrespective of increase or decrease in the mean values. All the treatments, except a few, had shown negative shift in the mean panicle length over the control. The maximum negative shift in the mean panicle length over the control in Taraori Basmati was observed at 20 kR gamma rays + 1.5 mM SA in M2 and M3 generations. In case of Pusa Basmati 1, the maximum negative shift in the mean panicle length over the control was observed at 0.05 M EMS treatment in M2 and M3 generations. In general, there was a wider range in the treated population as compared to the control in both the varieties in both the generations [27].

Table 1: Mean, range and coefficient of variation (CV) for plant height in Basmati rice in M2 and M3 generations

Treatment	M2 generation				M3 generations			
	Taraori Basmati		Pusa Basmati 1		Taraori Basmati		Pusa Basmati 1	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Gamma rays								
5 kR	141.20	6.61	98.20	5.43	142.90	5.37	99.45	4.71
10 kR	142.00	7.63	99.42	5.73	143.45	6.41	100.85	4.02
15kR	144.80**	8.10	101.31	6.04	146.25	7.01	103.00	6.14
20 kR	138.48**	9.14	95.11**	6.19	140.00**	8.94	96.75**	6.70
25 kR	144.90*	10.82	101.32	6.46	146.20**	9.56	102.60	6.75
30 kR	142.05	10.14	99.06	7.60	143.45	9.81	100.70	7.82
EMS								
0.02 M	138.45**	7.00	97.21	5.78	140.00	6.65	98.65	4.17
0.03 M	138.18**	8.05	95.41	6.40	139.35**	7.86	96.70**	5.70
0.04 M	144.85**	9.28	101.21	7.35	146.05**	8.03	102.90	6.71
0.05 M	142.00	10.41	99.10	7.45	143.35	9.01	100.55	6.85
Gamma rays + EMS								
10 kR + 0.03 M	142.10	7.75	99.16	6.14	143.40	6.45	100.70	5.16
15kR + 0.03 M	142.20	9.55	99.21	6.42	143.45	8.21	101.00	5.03
20 kR+ 0.03 M	141.95	10.43	98.05	7.73	143.20	9.98	99.45	6.06
10 kR+ 0.04 M	139.92*	7.83	96.42**	5.53	141.05	6.56	97.70**	4.92
15kR+ 0.04 M	146.02**	10.93	103.22**	6.75	147.35**	9.57	104.50**	5.03
20 kR+ 0.04 M	142.00	10.57	99.56	8.31	143.25	9.08	100.85	7.57
SA								
0.5 mM	138.25**	7.77	95.73**	5.52	139.75**	6.58	96.90**	4.88
1.0 mM	138.45**	9.42	95.50**	6.09	139.55**	8.17	96.70**	5.20
1.5 mM	138.48**	10.98	95.40**	6.62	139.90**	9.69	96.60**	6.64
2.0 mM	141.75	10.78	98.50	7.03	143.10	9.54	99.90**	6.84
Gamma rays + SA								
10 kR + 1.0 mM	140.25	7.15	95.40**	6.19	141.65	6.81	96.75**	5.09
15kR + 1.0 mM	142.05	8.82	99.41	6.17	142.85	7.52	100.90	50.87
20 kR+1.0 mM	138.30**	1.78	95.31**	7.82	139.75**	9.31	96.80**	6.25
10 kR+ 1.5 mM	141.55	8.08	98.25	6.30	142.95	7.78	99.80	5.31
15kR+ 1.5 mM	145.75**	10.28	102.25**	7.93	147.00**	9.89	104.00**	6.92
20 kR+1.5 mM	141.85	10.15	98.20	8.06	143.10	9.74	99.75	7.17
Gamma rays + EMS + SA								
5 kR + 0.02 M + 0.5 mM	138.15**	6.98	95.70**	6.21	140.00	5.68	96.90**	5.19
Control	142.50	5.16	99.51	4.36	143.75	5.24	100.73	4.75
SE±	1.093		1.087		1.147		1.186	

Table 2: Mean, range and coefficient of variation (CV) for number of panicle bearing plant in Basmati rice in M2 and M3 generations

Treatment	M2 generation				M3 generations			
	Taraori Basmati		Pusa Basmati 1		Taraori Basmati		Pusa Basmati 1	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Gamma rays								
5 kR	7.10	21.42	7.90	72.83	7.18	19.49	7.95	25.43
10 kR	7.00	19.32	7.80	23.98	7.09	18.41	7.89	21.88
15kR	5.4**	27.11	6.90**	25.86	5.60**	22.12	7.01*	24.74
20 kR	7.15	20.58	7.95	27.78	7.23	19.43	8.05	26.53
25 kR	5.25**	27.37	6.05**	23.73	5.38**	24.22	6.25**	21.25
30 kR	4.3**	25.62	6.10	22.52	5.49	23.12	6.18**	20.13
EMS								
0.02 M	7.02	27.49	7.85	28.20	7.25	23.25	7.90	25.30
0.03 M	5.8**	20.48	6.60**	25.81	5.90**	19.55	6.65**	24.50
0.04 M	7.10	21.91	7.90	25.76	7.21	20.88	7.98	23.46
0.05 M	5.9**	18.81	6.70**	22.53	6.00**	17.88	6.77**	21.41

Table 2: Continued

Gamma rays + EMS								
10 kR + 0.03 M	7.15	24.81	7.95	27.52	7.23	22.43	8.02	24.21
15kR + 0.03 M	6.10**	21.60	6.90**	28.67	6.18**	20.43	6.97*	27.35
20 kR+ 0.03 M	7.95	23.02	8.75	27.02	8.00	22.03	8.79	25.31
10 kR+ 0.04 M	7.10	23.08	7.90	27.02	7.19	22.15	8.87*	26.12
15kR+ 0.04 M	5.90**	26.98	8.80	28.09	8.09	25.25	6.85**	26.32
20 kR+ 0.04 M	8.00	24.54	6.70**	25.29	6.00**	23.42	7.97	23.98
SA								
0.5 mM	7.00	20.31	7.80	28.91	7.12	20.12	7.93	26.85
1.0 mM	7.20	24.00	8.00	26.87	7.31	23.51	8.09	24.31
1.5 mM	5.25**	17.83	6.05**	23.89	5.38**	17.50	6.25**	22.72
2.0 mM	7.90	22.03	8.70	21.80	7.97	21.03	8.90**	20.18
Gamma rays + SA								
10 kR + 1.0 mM	7.05	26.66	7.85	29.82	7.25	23.25	7.98	27.32
15kR + 1.0 mM	7.10	19.60	7.90	29.55	7.19	19.50	8.07	28.05
20 kR+1.0 mM	7.89	20.97	8.42	28.67	7.99	20.61	8.92*	27.63
10 kR+ 1.5 mM	5.20**	17.17	6.00**	29.20	5.40**	17.10	6.20**	25.39
15kR+ 1.5 mM	8.02*	19.16	8.82*	26.90	8.18*	18.19	8.85*	23.25
20 kR+1.5 mM	5.15**	17.25	6.85**	26.79	5.35**	17.10	6.95*	23.12
Gamma rays + EMS + SA								
5 kR + 0.02 M + 0.5 mM	6.50	16.81	7.30	27.79	6.68	15.95	7.48	26.21
Control	7.20	16.69	8.00	21.69	7.23	17.01	7.98	22.70
SE±	0.410		0.417		0.484		0.432	

Table 3: Mean, range and coefficient of variation (CV) for 100 seed weight in Basmati rice in M2 and M3 generations

Treatment	M2 generation				M3 generations			
	Taraori Basmati		Pusa Basmati 1		Taraori Basmati		Pusa Basmati 1	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Gamma rays								
5 kR	2.51	3.26	2.19	5.02	2.55	2.89	2.19	4.98
10 kR	2.50	3.48	2.19	5.09	2.54	2.92	2.20	4.82
15kR	2.21	5.12	1.88**	6.06	2.25**	4.82	1.90**	5.18
20 kR	2.51	5.42	2.19	6.31	2.55	4.48	2.19	5.93
25 kR	2.21	6.20	1.93**	7.41	2.24**	5.92	1.97**	7.12
30 kR	2.26	6.42	1.83**	6.98	2.29**	6.12	1.85**	6.19
EMS								
0.02 M	2.52	4.31	2.19	4.85	2.54	3.88	2.19	4.14
0.03 M	2.35	4.52	1.82**	5.61	2.38**	3.79	1.84**	5.30
0.04 M	2.50	5.22	2.19	6.12	2.54	4.29	2.19**	5.98
0.05 M	2.21	6.31	1.87**	6.98	2.26**	5.82	1.89**	6.44
Gamma rays + EMS								
10 kR + 0.03 M	2.52	3.81	2.18	6.42	2.55	3.62	2.19	6.12
15kR + 0.03 M	2.21	4.28	1.89**	7.18	2.24**	3.14	1.89**	7.01
20 kR+ 0.03 M	2.66	4.44	2.23	5.92	2.68**	4.32	2.25	5.70
10 kR+ 0.04 M	2.51	3.38	2.19	5.88	2.58	3.17	2.18	5.46
15kR+ 0.04 M	2.69	3.46	2.39**	4.65	2.70**	3.28	2.39**	5.46
20 kR+ 0.04 M	2.21	5.02	1.88**	6.82	2.24**	4.98	1.89**	6.36
SA								
0.5 mM	2.52	4.32	2.18	7.48	2.54	3.87	2.20	7.21
1.0 mM	2.50	5.22	2.18	5.38	2.55	4.49	2.19	2.15
1.5 mM	2.20	5.41	1.88**	5.48	2.25**	5.02	1.89**	5.21
2.0 mM	2.69	5.32	2.26	7.04	2.70**	5.10	2.28	6.82
Gamma rays + SA								
10 kR + 1.0 mM	2.51	3.81	2.19	7.12	2.56	3.42	2.19	5.91
15kR + 1.0 mM	2.52	4.22	2.28	7.41	2.56	3.81	2.29	7.01
20 kR+1.0 mM	2.66	5.62	2.19	5.31	2.68**	4.79	2.19	6.91
10 kR+ 1.5 mM	2.22	3.81	1.88**	6.62	2.25**	3.32	1.89**	5.11
15kR+ 1.5 mM	2.71	5.42	2.27	6.60	2.71**	4.82	2.29	6.13
20 kR+1.5 mM	2.20	6.41	1.88**	6.60	2.22**	5.02	1.88**	6.13
Gamma rays + EMS + SA								
5 kR + 0.02 M + 0.5 mM	2.52	4.82	2.19	5.82	2.55	4.21	2.19	5.41
Control	2.51	3.08	2.18	4.02	2.52	3.12	0.06	4.12
SE±	0.057		0.055		0.058		0.058	

Table 4: Mean, range and coefficient of variation (CV) for grain yield per plant Basmati rice in M2 and M3 generations

Treatment	Taraori Basmati		Pusa Basmati 1		Taraori Basmati		Pusa Basmati 1	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Gamma rays								
5 kR	10.15	32.43	12.37	29.52	10.45	30.52	12.70	28.51
10 kR	9.97	33.56	12.19	30.47	10.05	31.33	12.45	29.23
15kR	8.24**	37.43	10.28**	35.61	8.50**	35.23	10.40**	31.22
20 kR	10.29	37.41	12.52	34.52	9.65	34.38	12.78	32.11
25 kR	7.64**	39.33	9.21**	36.40	7.80**	37.32	9.25**	33.41
30 kR	7.86**	39.08	9.96**	36.72	7.95**	36.28	10.25**	33.25
EMS								
0.02 M	9.90	28.13	12.10	24.55	9.98	27.42	12.45	22.23
0.03 M	7.59**	37.33	10.71**	34.00	7.65**	34.28	10.95**	31.23
0.04 M	10.04	36.21	12.25	33.88	10.40	33.31	12.70**	31.78
0.05 M	7.19**	39.23	9.24**	36.37	7.35**	38.25	9.75**	33.25
Gamma rays + EMS								
10 kR + 0.03 M	10.10	31.01	12.24	27.16	10.25	30.26	12.50	26.25
15kR + 0.03 M	7.83**	32.81	9.93**	29.51	7.95**	31.72	10.05**	28.24
20 kR+ 0.03 M	11.91	35.32	11.30	32.81	11.40	33.28	13.60	30.62
10 kR+ 0.04 M	10.11	28.72	12.33	24.66	9.45	26.58	12.60	22.23
15kR+ 0.04 M	11.32	32.81	13.74	29.21	11.45	30.75	13.90	26.31
20 kR+ 0.04 M	7.28**	37.32	8.34**	33.20	7.43**	35.21	9.90**	30.13
SA								
0.5 mM	9.90	31.01	12.11	28.17	10.10	30.81	13.75	26.25
1.0 mM	10.22	33.50	12.44	29.09	9.30	29.01	12.95	27.15
1.5 mM	7.43**	36.72	8.47**	32.49	7.58**	30.77	9.95	31.02
2.0 mM	10.79	36.92	12.18	33.30	10.85	34.88	12.60	31.12
Gamma rays + SA								
10 kR + 1.0 mM	9.94	32.53	12.14	27.02	10.00	30.52	12.70	26.15
15kR + 1.0 mM	10.25	33.53	12.44	30.99	10.35	31.32	12.60	28.10
20 kR+1.0 mM	11.01	38.31	13.60	34.65	10.18	36.11	14.80	31.25
10 kR+ 1.5 mM	7.29**	32.32	9.26**	27.79	7.45**	30.22	9.70**	26.25
15kR+ 1.5 mM	11.31	33.51	13.72	29.79	11.98	31.11	13.96	26.25
20 kR+1.5 mM	7.43**	38.21	8.49**	29.31	7.55**	35.12	9.75**	28.15
Gamma rays + EMS + SA								
5 kR + 0.02 M + 0.5 mM	9.36	31.81	11.58	29.66	9.75	30.25	12.05	28.15
Control	10.54	27.80	12.76	24.94	10.68	36.92	12.82	25.12
SE±	0.818		0.677		0.723		0.808	

Number of Panicle Bearing Tillers per Plant:

The data revealed that there was slight change in the magnitude of range for number of panicle bearing tillers per plant in both the varieties in both the generations. The positive and negative shifts in the mean over the control were observed in both the varieties. In case of Taraori Basmati, the negative shift was maximum due to combination treatment of 20 kR gamma rays + 1.5 mM SA in both the generations. The higher doses of mutagenic treatments exhibited wider range for this trait. In case of Pusa Basmati 1, the maximum reduction in the number of panicle bearing tillers per plant was observed at 10 kR gamma rays + 1.5 mM SA and 30 kR gamma rays in M2 and M3 generations, respectively. In general, the CV among treated population was higher than the control in both the generations.

100-seed weight was observed in both the varieties in both the generations at many treatment doses (Table 3). The maximum increase in 100-seed weight in Taraori Basmati was observed in M2 and M3 generations at 15 kR gamma rays + 1.5 mM SA. The CV of the treated population in general was higher than the control in both the varieties in both the generations. In case of Pusa Basmati 1 an increase in shift was maximum at 15 kR gamma rays + 0.04 M EMS while a reduction in the mean was extreme at 0.03 M EMS in both the generations.

It was inferred from the tables 1 -6 that various doses and / or concentrations of gamma rays, EMS and SA used singly or in combination were quite effective in inducing genetic variability for yield and yield traits in M2 and M3 generations in Basmati rice. However, 15 kR, 25 kR and 30 kR doses of gamma rays, 0.03 M EMS, 1.5 mM SA,

15 kR gamma rays + 0.03 M EMS, 20 kR gamma rays 0.01 M EMS, 10 kR gamma rays + 1.5 mM SA and 20 kR gamma rays + 1.5 mM SA were found to be more effective in inducing genetic variability. From the practical breeding point of view, the induced variations assumed a greater scope for the improvement of this crop. The range of variation as shown by different mutagenic treatments was in general wider than the respective controls for all the quantitative traits in Taraori Basmati and Pusa Basmati 1 in M2 and M3 generations. The experimental findings clearly demonstrated a difference in the mean values of various quantitative traits in the treated population from their respective controls. The majority of the mutagenic treatments induced negative shift in the mean away from the control for yield and yield traits in M2 generation in both the varieties. However, only a few treatments induced significant positive shift in the M2 generation. The mean values of many treatment were found comparable to the respective controls in M2 generation in both the varieties. Conclusively, the mean values for different traits shifted either to positive or negative direction away from the control due to mutagenic treatments. Similar findings have been observed in bread wheat [28-31], soyabean, urdbean and grass pea. The mean performance exhibited improvement in most of the mutagenic treatments in M3 generation as compared to the corresponding treatments in M3 generations. Similar findings were also reported in rice [33,34].

The experimental findings under reference suggested that the induced variability measured as coefficient of variation increased in the treated population as compared to the respective controls of these traits in both the varieties. In general, it was found that the relationship between increase in variability and doses was not linear. [35] reported a similar case for lack of linearity that might be due to elimination of mutations through gametic or zygotic selection that eliminates a large number of new genotypes from the population soon after following mutagenic treatments and thus reduced potential genetic variability. The magnitude of variability was generally lower in the mutagenic treatment in M3 generation than the corresponding treatments in M2 generation in both the rice varieties with few exceptions. The probable reasons for a decline in variability in M3 in comparison to M2 may be attributed to the tendency approaching homozygosity [36]. [37] suggested that the decrease in variability in M3 generation occurred due to increase in the frequency of genetic deaths due to homozygosity of the harmful genes in M3 generation.

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