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Induced polygenic variability in M_2 generation and its relationship with production of high-yielding mutants in finger millet

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Abstract

Seeds of two finger millet varieties, VR 708 and GPU 26 were treated with three doses each of gamma rays (150, 300 and 450 Gy), ethyl methane sulphonate (0.15, 0.30 and 0.45%) and nitroso guanidine (0.015, 0.030 and 0.045%) in addition to two combination treatments of gamma rays 300 Gy + EMS 0.30% and gamma rays 300 Gy + NG 0.030%. The M_1 generation was harvested as treatment bulk and the M_2 to M_4 generations were raised. In M_2 generation, most treatment populations exhibited reduction in population mean and increase in population variance for all the six traits studied and the magnitude of such changes varied with mutagens, their doses and the variety. In general, greater shift in mean and variance was observed in treatments with higher doses of NG and EMS in case of VR 708 and higher doses of NG and combination treatments in case of GPU 26. Most mutagen treated populations showed wider range of variation than the parent variety and the variation was in both directions. Genetic advance estimates showed that selection in many M_2 treatment populations would be effective in bringing about improvement in yield/plant and its direct components like tillers/plant, fingers/ear and finger length. Following selection among M_2 plants and M_3 progenies on the basis of higher yield, eight high yielding mutant cultures in VR 708 and nine mutants in GPU 26 were isolated in M_4 generation. Vast majority of the high yielding M_3 progenies and M_4 mutant cultures were from the groups of M_2 mutagenic treatments showing significantly higher population variance for yield/plant. Thus, selection of high-yielding M_2 plants and M_3 progenies in mutagenic treatments with much increased M_2 variance for yield would be effective in isolation of high yielding micromutant cultures.

Key words: Finger millet, polygenic variability, genetic parameters, mutagenic treatments, micromutants

Introduction

The productivity level of finger millet (*Eleusine coracana* (2n = 4x = 36) Gaertn.) needs improvement by evolving high yielding varieties, which depends on the availability of variability for yield and its component characters in the population. Induction of mutations by using physical and chemical mutagens may be necessary and helpful to generate new variability [1-2]. Information on the quantum of induced polygenic variability or micrornutations and the genetic parameters for different polygenic traits in M_2 generation gives an indication about the scope of improvement in the traits through selection [1, 3, 4]. In the present study attempt has been made to ascertain the magnitude of induced genetic variability and the genetic parameters of yield and its components in M_2 generation along with the relationship of this variability in isolating high yielding mutant progenies/cultures in M_3 and M_4 generations.

Materials and methods

Seeds of two varieties of finger millet i.e., VR 708 and GPU 26 were administered mutagenic treatments with three doses each of gamma rays (150, 300 and 450 Gy), ethyl methane sulphonate (0.15, 0.30 and 0.45%) and nitroso guanidine (0.015, 0.030 and 0.045%) employed singly or in combination. The nine single mutagenic treatments of gamma rays, EMS and NG were coded as G1, G2, G3, E1, E2, E3 and M1, N2, N3, respectively. The two combination treatments were gamma rays 300 Gy + EMS 0.30% and gamma rays 300 Gy + NG 0.030% coded as GE2 and GN2, respectively. Dry seeds were irradiated with gamma rays at Bhava Atomic Research Centre, Trombay. For treatment with EMS and NG, the seeds were pre-soaked in distilled water for 10 hours and then treated with their aqueous solutions for 6 hours. For combination treatments, seeds were first irradiated with gamma rays 300 Gy and then treated with EMS 0.30% or NG 0.030% solution. After treatment, the seeds were thoroughly washed with running water and then dried on blotting paper.

The M_1 generation was grown and harvested as treatment bulk. The M_2 to M_4 generations were raised during *kharif* seasons of 2002-2004 at Orissa University of Agriculture and Technology, Bhubaneswar. In M_2 generation, two separate trials, one for each variety were laid out in RBD with three replications with a spacing of 30 x 10 cm. Different types of chlorophyll and morphological macromutants were identified and harvested separately. For study of induction of micromutations, observations on 30 randomly selected normal looking plants from each treatment in each replication were recorded on five yield attributing traits and yield/plant. Mean and variance of the traits in each treatment population were estimated and subjected to statistical analysis. The genetic parameters like GCV, heritability (h^2) and genetic advance (GA) were estimated [5]. Fifteen M_2 plants (16.7% selection intensity) from each of eleven mutagenic treatments were selected on the basis of higher yield/plant [6] and used to grow the M_3 generation. Fourty four high yielding mutant progenies were selected in M_3 based on yield/plant and were evaluated in M_4 generation. High yielding mutant cultures were also identified in M_4 generation on the basis of yield/plant.

Results and discussion

In order to assess the nature and magnitude of induced polygenic variability or micromutations in traits like plant height, tillers/plant, fingers/ear, finger length, ear weight/plant and yield/plant, the different mutagenic treatment populations in M_2 generation were evaluated through statistical parameters such as mean and variance. Analysis of variance of M_2 population means and variances for the traits showed significant differences among the treatments for all the characters except population means of finger length in both the varieties.

Almost all mutagen treated M_2 populations showed varied extent of negative shift in mean for all the characters studied in both the varieties, and the shift was significant in most cases (Tables 1 and 2). However, the magnitude of shift in mean varied with the mutagens, their concentrations, parental genotypes and the character under consideration. This negative shift in mean was more conspicuous for plant height in GPU 26 and tillers/plant, fingers/ear and yield/plant in both the varieties, and it was the least for finger length. Similar differential negative shift of mean in different M_2 populations were reported earlier in finger millet [1-3]. In most of the above reports, the shift of mean varied with mutagens and their doses. In this study, there was greater reduction in higher doses for most traits. A comparative study of the effect of mutagens indicated that the negative shift of mean was more pronounced in NG and combination treatments in both the varieties. This might be due to the drastic mode of action of NG and induction of more mutations with negative effects [2, 7-9]. Lower magnitude of negative shift in gamma rays and EMS treatments might be due to less drastic effect and induction of mutations in either direction [8, 10]. The negative shift could be attributed to either physiological damage caused chiefly by chemical mutagens or chromosomal aberrations caused mainly by irradiations [7, 9]. Induction of more chromosomal aberrations was reported in NG than EMS treatment [11].

All the mutagen treated M_2 populations showed varied extent of increase in population variance than control population for all the six characters studied (Table 1 and 2). However, the magnitude of increase in population variance varied with the mutagen, their concentration, parental genotypes and the character under consideration. For most characters up to two-fold increase in variance was observed in certain treatments. Similar. results had earlier been reported in finger millet [1, 3]. The study showed that though dose-variance relationship was not completely linear, in most of the cases higher doses of mutagens induced greater variance. Among the mutagens, NG and combination treatments induced more variability in both the varieties. Higher effectiveness of the alkylating agents in inducing polygenic variability could be explained on the fact that they produce mostly point mutations in comparison to gamma rays that induces higher proportion of chromosomal aberrations. Rapoport [12] described the mutagens belonging to the nitroso group as “super mutagens” in view of their higher mutagenic effects, a consequence of their alkylating ability on the gene directly.

The M_2 population variance *per se* does not give the true picture as it includes the genetic component of induced genetic variability due to mutagenic treatment (GCV) and environmental component of variability (ECV). Depending upon the magnitude of induced genetic variability in different treatment populations the genetic parameters like heritability and genetic advance under selection would vary and these parameters can give an indication about the effectiveness of mutagenic treatments for induction of micromutations and scope of improvement for the traits through selection. In the present study, range, GCV, heritability and genetic advance in M_2 populations were estimated for important

yield components like tillers/plant, fingers/ear and finger length as well as for yield/plant (Tables 1 and 2).

Most mutagenic treatments induced wider range of variation in M_2 populations in both directions for all the four traits. The GCV estimates varied with mutagenic treatments and were in general, moderately high for tillers/plant and yield/plant, and low for other traits in both the varieties. Heritability estimates for different traits varied with mutagens and their doses and were relatively of higher magnitude in VR 708 than GPU 26. In certain cases, the estimates were relatively high being up to 54% in VR 708, indicating greater scope for selection. Genetic advance under selection (5% selection intetreatments and characters studied. The study revealed that selection in treated populations might lead to an improvement of up to 0.95 and 0.72 tillers/plant, 1.69 and 1.00 fingers/ear, 0.71 and 0.85 cm in finger length and 3.26 and 2.09 g in yield/plant in certain treatments of VR 708 and GPU 26, respectively. Genetic advance as percentage of mean also increased in the treatments and was comparatively higher for tillers/plant, fingers/ear and yield/plant in both the varieties. Similar results for different traits were also reported earlier in sesame [4]. Simultaneous consideration of all the genetic parameters of the yield promoting traits indicated that the treatments G2, E3, N2, N3 and both the combination treatments (GE2 and GN2) appeared to be most effective for induction of micromutation in yield component traits and selection in M_2 population of these treatments would be effective in developing high yielding lines.

The magnitude and direction of induced polygenic variation in a particular trait would greatly determine the scope of selection of micromutants with improvement in the trait. Some earlier workers [13, 14] selected M_2 families showing increase in variance with increase or no change in mean for the trait and were successful in not only reducing the bulk material from early generation, but also isolated several micromutants in the later generations with improvement in the traits. With this rationale, both M_2 population mean and variance were assessed simultaneously in the present study and the mutagenic treatments of the two varieties are presented in two-dimensional graphs using M_2 population mean and population variance of yield/plant (Figs. 1 and 2). Though most treatment populations showed reduction in mean and increase in variance in comparison to control in Ma, the magnitude of such change varied with mutagens, their doses and with the variety. The nature and magnitude of such changes may have bearing on the scope of improvement through selection. The significance of changes in mean and variance from the parental population for yield/plant was tested using CD at 5% and classified using (i) parental (C) population mean - CD and (ii) parental (C) population variance + CD. On this basis, the mutagen treated populations of both the varieties were classified into four groups: No significant decrease in mean with no significant increase in variance (Group I) or with significant increase in variance (Group II) and significant decrease in mean with significant increase in variance (Group III) or with no significant increase in variance (Group IV). Constant selection pressure based on yield was applied to all the treatments for selection of M_2 plants and M_3 families and evaluated in M_4 generation. The frequency of superior mutant progenies/cultures with higher yield than the parent variety obtained in M_3 and M_4 generations from different M_2 mutagenic treatment groups are given in Table 3.

The Group I mutagenic treatments (G1, E1, N1 of VR 708 and G1 of GPU 26) showing no significant decrease in mean and no significant increase in variance were supposed to possess very little or no induction of micromutations in yield. Thus, selection from these treatments was expected to have very little success. The study also revealed that only a small portion of high yielding M_3 progenies and no high yielding mutant culture in M_4 of both the varieties were obtained from this group of treatments, thus, confirming the expectations. The Group IV (no treatment of VR 708 and only G3 of GPU 26) showing significant decrease in mean without significant increase in variance would indicate that induction of micromutation might be non-random, mostly in negative direction. Induction of such micromutations only with negative effect is generally rare, particularly in yield. The scope of improvement through selection from this group would be quite restricted. The present study also revealed the production of very few M_3 progenies or no high yielding M_4 culture from this group of treatments.

The Group III treatments (E3, N2, N3, GN2 of VR 708 and E3, N1, N2, N3, GN2 of GPU 26) showing significant increase in variance with significant decrease in mean were supposed to have more plants with induction of micromutations having negative effects and relatively small proportion of plants with negative effects. Thus, micromutation induction in yield in this group appeared to be more frequent in the opposite direction of previous selection history of the parental genotype and less in positive direction. In spite of that, adoption

Table 1. Parameters of genetic variability for different quantitative traits in mutagenic treatments of finger millet variety VR 708 in M_2 generation

Treatment	Tr. code	Range	Mean	Vari-ance	GCV (%)	h^2 (%)	GA (5%)	GA (% of mean	Range	Mean	Vari-ance	GCV (%)	h^2 (%)	GA (%)	GA (% of mean
		Tillers/plant							Fingers /ear						
Gamma rays															
150 Gy	G1	1-5	1.94	0.67	18.6	19.40	0.33	17.0	4-10	7.17	2.36	10.4	23.73	0.75	10.5
300 Gy	G2	1-4	1.80	0.80*	28.3	32.50	0.60	33.3	4-11	7.08	3.00*	15.5	40.00	1.43	20.2
450 Gy	G3	1-6	1.73*	0.99*	38.8	45.45	0.93	53.8	3-12	6.78*	2.77*	14.5	35.02	1.20	17.7
EMS															
0.15%	E1	1 -5	1.89	0.57	9.2	5.26	0.08	4.2	4-11	7.50	2.42	10.5	25.62	0.82	10.9
0.30%	E2	1-4	1.72*	0.63	17.4	14.29	0.23	13.4	4-11	6.85*	2.39	11.2	24.69	0.79	11.5
0.45%	E3	1-4	1.57*	0.87*	36.6	37.93	0.73	46.5	4-10	6.71*	2.79*	14.8	35.48	1.22	18.2
NG															
0.015%	N1	1-6	1.92	0.76	24.4	28.95	0.52	27.1	3-10	7.35	2.12	7.7	15.09	0.45	6.1
0.030%	N2	1-6	1.70*	0.99*	39.5	45.45	0.93	54.7	3-10	6.68*	6.67*	13.9	32.58	1.10	16.5
0.045%	N3	1-5	1.61*	0.93*	38.8	41.94	0.83	51.6	3-11	6.61*	3.02*	16.7	40.40	1.45	21.9
Combinations															
Gamma rays 300 Gy + EMS 0.30%	GE2	1-4	1.87	1.00*	36.3	46.00	0.95	50.8	3-12	7.01	3.29*	17.4	45.29	1.69	24.1
Gamma rays 300 Gy+NG 0.030%	GN2	1-4	1.70*	0.89*	34.8	39.33	0.76	44.7	3-11	6.95*	2.56*	12.5	29.69	0.98	14.1
Parent	C	1-4	1.96	0.54	-	-	-	-	4-9	7.62	1.80	-	-	-	-
CD (5%)		-	0.22	0.25		-	-	-	-	0.62	0.63	-	-	-	-
		Finger length (cm)							Yield/plant (g)						
Gamma rays															
150Gy	G1	3.0-6.4	5.64	0.28	3.5	14.29	0.16	2.8	2.1-17.4	6.31	5.38	16.5	20.26	0.97	15.4
300 Gy	G2	4.0-6.5	5.49	0.32	5.2	25.00	0.29	5.3	1.7-16.1	6.03	6.60*	25.2	35.00	1.85	30.7
450 Gy	G3	4.0-7.0	5.51	0.42*	7.7	42.85	0.57	10.3	1.0-18.4	6.12	7.07*	27.2	39.32	2.15	35.1
EMS															
0.15%	E1	4.0-7.0	5.65	0.28	3.5	14.29	0.16	2.8	1.7-14.7	6.22	4.57	8.5	6.13	0.27	4.3
0.30%	E2	4.0-7.1	5.46	0.35*	6.1	31.43	0.38	6.9	2.1-18.3	6.40	6.91*	25.3	37.92	2.05	32.0
0.45%	E3	3.8-7.0	5.49	0.46*	8.5	47.83	0.67	12.2	1.4-16.0	5.88*	8.36*	34.3	48.68	2.90	49.3
NG															
0.015%	N1	4.0-6.6	5.42	0.40*	7.4	40.00	0.52	9.6	1.7-13.9	6.12	4.93	13.1	12.98	0.59	9.6
0.030%	N2	4.0-7.0	5.32	0.39*	7.3	38.46	0.49	9.2	1.2-15.3	5.94*	8.59*	34.9	50.05	3.02	50.8
0.045%	N3	4.0-6.8	5.18	0.48*	9.5	50.00	0.71	13.7	1.3-11.4	5.14*	9.45*	44.2	54.60	3.46	67.3
Combinations															
Gamma rays 300 Gy + EMS 0.30%	GE2	3.8-7.6	5.60	0.47*	8.6	48.94	0.69	12.3	1.6-16.2	6.02	6.95*	27.1	38.27	2.08	34.6
Gamma rays 300 Gy + NG 0.030%	GN2	4.0-7.0	5.33	0.37*	6.8	35.14	0.44	8.3	1.0-15.8	5.70*	9.12*	38.6	52.96	3.29	57.7
Parent	C	4.0-7.0	5.79	0.24	-	-	-	-	2.6-12.6	6.38	4.29	-	-	-	-
CD (5%)		-	NS	0.10	-	-	-	-	-	0.37	1.97	-	-	-	-

Significant decrease (in mean) or increase (in variance) over control at 5% level

Table 2. Parameters of genetic variability for different quantitative traits in mutagenic treatments of finger millet variety GPU 26 in M_2 generation

Treatment	Tr. code	Range	Mean	Vari-ance	GCV (%)	h^2 (%)	GA (5%)	GA (% of mean	Range	Mean	Vari-ance	GCV (%)	h^2 (%)	GA (%)	GA (% of mean
		Tillers/plant							Fingers /ear						
Gamma rays															
150 Gy	G1	1-5	1.73	0.67	15.3	10.45	0.18	10.4	4-8	6.19	1.08	7.0	17.5	0.38	6.1
300 Gy	G2	1-6	1.67*	0.83*	28.7	27.71	0.52	31.1	3-8	6.11	1.16	8.5	23.2	0.52	8.5
450 Gy	G3	1-4	1.64*	0.80*	27.3*	25.00	0.46	28.0	3-10	6.18	1.02	5.8	12.7	0.27	4.4
EMS															
0.15%	E1	1-5	1.75	0.74	21.4	18.92	0.34	19.4	3-8	6.15	1.10	7.5	19.0	0.41	6.7
0.30*%	E2	1-6	1.72	0.84*	28.5	28.57	0.54	31.4	3-9	6.12	1.26*	9.9	29.3	0.68	11.1
0.45%	E3	1 -4	1.64*	0.77	25.1	22.08	0.40	24.4	3-8	5.82*	1.37*	11.9	35.0	0.84	14.4
NG															
0.015%	N1	1-6	1.74	0.78*	24.4	23.08	0.42	24.1	3-8	5.90	1.20*	9.4	25.8	0.58	9.8
0.030%	N2	1-6	1.60*	0.94*	36.4	36.17	0.72	45.0	3-9	5.80*	1.31*	11.2	32.0	0.76	13.1
0.045%	N3	1-4	1.57*	0.88*	33.7	31.82	0.61	38.9	3-8	5.82*	1.33*	11.4	33.0	0.79	13.6
Combinations															
Gamma rays 300 Gy + EMS 0.30%	GE2	1-5	1.73	0.75	22.4*	20.00	0.36	20.8	3-9	5.84*	1.48*	13.2	39.8	1.00	17.1
Gamma rays 300 Gy + NG 0.030%	GN2	1-5	1.69*	0.94*	34.5	36.17	0.72	42.6	3-8	5.75*	1.35*	11.8	34.0	0.82	14.3
Parent	C	1-4	1.84	0.60	-	-	-	-	4-9	6.13	0.89	-	-	-	-
CD (5%)		-	0.13	0.18	-	-	-	-	-	0.25	0.31	-	-	-	-
		Tillers/plant							Fingers /ear						
Gamma rays															
150 Gy	G1	5.4-9.3	7.17	0.63	3.9	12.70	0.21	2.9	1.0-17.0	7.76	7.63	10.6	8.91	0.77	9.9
300 Gy	G2	5.0-11.9	7.26	0.75*	6.2*	26.67	0.48	6.6	1.6-13.6	7.65	9.67*	21.6	28.13	1.80	23.5
450 Gy	G3	5.2-9.4	7.03	0.73	6.0	24.66	0.43	6.1	1.1-17.5	7.11*	7.77	12.7	10.55	0.61	8.6
EMS															
0.15%	E1	5.5-11.0	7.46	0.54	1.3	1.85	0.03	0.0	1.8-17.6	7.75	8.37*	15.4	16.97	1.01	13.0
0.30%	E2	5.5-10.0	7.32	0.66	4.5	16.67	0.28	3.8	1.8-16.1	7.74	8.80*	17.6	21.02	1.28	16.5
0.45%	E3	5.2-9.3	7.36	0.89*	7.9	38.20	0.74	10.1	1.3-20.1	7.24*	8.63*	17.9	19.47	1.18	16.3
NG															
0.015%	N1	5.2-9.7	7.29	0.79*	6.7	30.38	0.56	7.7	1.6-17.3	7.40*	9.35*	20.9	25.67	1.62	21.9
0.030%	N2	4.5-10.0	7.01	0.85*	7.8	35.29	0.67	9.6	1.8-20.1	7.52*	10.18*	23.9	31.73	2.09	27.8
0.045%	N3	5.7-9.8	7.01	0.95*	9.0	42.11	0.85	2.1	2.3-17.0	6.81*	8.43*	17.9	17.56	1.05	15.4
Combinations															
Gamma rays 300 Gy + EMS 0.30%	GE2	5.7-9.8	7.33	0.77*	6.4	28.57	0.52	7.1	1.1-17.3	7.61	8.25*	15.0	15.76	0.93	12.2
Gamma rays 300 Gy + NG 0.030%	GN2	5.5-10.2	7.19	0.95*	8.8	42.11	0.85	1.8	1.2-16.4	6.96*	8.86*	19.9	21.56	1.32	19.0
Parent	C	5.5-9.5	7.77	0.55	-	-	-	-	3.4-15.6	8.11	6.95	-	-	-	-
CD (5%)		-	NS	0.19	-	-	-	-	-	0.57	1.15	-	-	-	-

Significant.decrease (in mean) or increase (in variance) over control at 5% level

Table 3. Classification of mutagenic treatments into different groups based on M_2 mean and variance for yield/plant and frequency of high yielding mutant progenies/cultures obtained from them in M_3 and M_4 generations

Mutagenic treatment group in M_2	Mutagenic treatments	High yielding mutant progenies/cultures			
		M_3		M_4	
		Number	%	Number	%
VR708					
Group I	G1,E1,N1,C	10	16.4	0	0.0
Group II	G2, G3, E2, GE2	29	47.5	4	50.0
Group III	E3, N2, N3, GN2	22	36.1	4	50.0
Group IV		0	0.0	0	0.0
		61	8		
GPU 26					
Group I	G1,C	5	7.7	0	0.0
Group II	G2, E1.E2, GE2	24	36.9	5	55.6
Group III	E3,N1,N2,N3,GN2	31	47.7	4	44.4
Group IV	G3	5	7.7	0	0.0
		65		9	

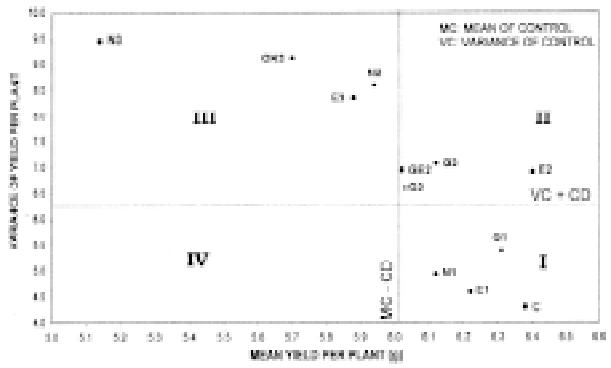

Fig. 1. Scatter diagram of the treatments in VR 708 with respect to mean and variance in M_2 generation

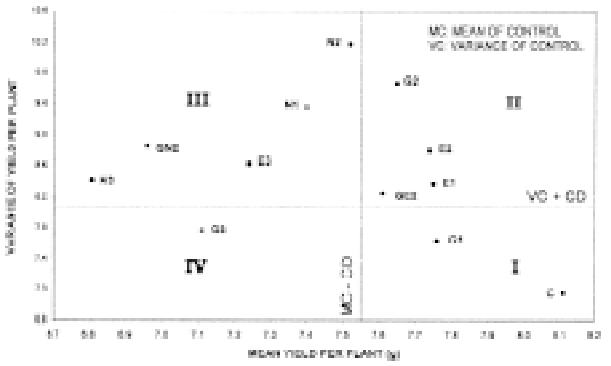

Fig. 1. Scatter diagram of the treatments in GPU 26 with respect to mean and variance in M_2 generation

of proper selection procedure may help in identification of relatively good number of high yielding mutants from these treatments. In the present study, this group produced reasonably large portion of high yielding M_3 progenies (36.1 and 47.7%) and M_4 cultures (50.0 and 44.4%) in VR 708 and GPU 26, indicating that selection in these treatments was effective in isolation of high yielding mutants.

The Group II showing significant increase in population variance for yield/plant without any significant decrease in treatment means included G2, G3, E2 and GE2 treatments in case of VR 708 and G2, E1, E2 and GE2 in case of GPU 26. This indicated that induction of micromutation in the trait in these treatments was random and in both positive and negative directions. The high yielding mutant progenies /cultures identified from this group of treatments in M_3 and M_4 generations were comparatively high i.e., 47.5 and 50% in case of VR 708 while 36.9% and 55.6% in case of GPU 26, respectively. Thus, the expectation of isolating more high yielding micromutants through selection from these treatments seems correct. Similar success in micromutational improvement through selection in M_2 families showing increased variability without decrease in mean with respect to parent and further selection of plants or lines have been reported earlier [2, 6]. In both the varieties, selection of M_2 plants and M_3 progenies in Group II mutagenic treatments was most effective in

isolation of high yielding mutants in M_4 generation. Similar selection in Group III treatments was also effective to little lesser extent and that in Group I and IV treatments was not much effective. Thus, it can be inferred that selection of high yielding M_2 plants and M_3 progenies in mutagenic treatments showing much-increased M_2 population variance for yield would be effective in isolation of high yielding micromutant lines.

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