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Efficacy of mutagenic treatments in producing useful mutants in finger millet (*Eleusine coracana* Gaertn.)

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Abstract

A mutation breeding project was initiated with finger millet varieties, VR 708 and GPU 26 using 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%) coupled with combination treatments of 300 Gy gamma rays with 0.30% EMS or 0.030% NG. Fifteen selected M_2 plant progenies from each of the eleven mutagenic treatments along with the parental variety were evaluated in M_3 generation. Four high yielding M_3 progenies from each treatment along with the parental variety were evaluated for yield and eight component traits in M_4 generation. In M_3 generation, of the 165 progenies, 61 in VR 708 and 65 in GPU 26 produced significantly higher yield than the parent and EMS treatments produced more of such high yielding progenies. In M_4, out of 44 progenies in each of VR 708 and GPU 26, 8 and 9 progenies showed superiority over the parental variety in one or more traits, respectively. High frequency of positive mutations was observed for 1000-grain weight, finger length and fingers/ear in case of VR 708 and fingers/ear and finger length in case of GPU 26. Moreover, EMS treatments produced more superior mutants (28.93% in VR 708 and 39.13% in GPU 26) in different traits than the other mutagenic treatments. Among the mutagenic treatments, the frequency of high yielding progenies in M_3 and M_4 generations were higher in 0.30% and 0.45% EMS, 0.030% NG and combination treatment of 300 Gy gamma rays + 0.30% EMS.

Key words: Finger millet, induced mutations, superior micromutants, mutagenic treatments

Introduction

Induced mutation has been perceived as an important tool to create additional variability for quantitative and qualitative traits in a number of crop plants [1]. Mutation breeding has already made significant contribution to crop improvement all over the world. This is amply evident from the fact that more than 2250 varieties of different crops had been released that were derived as direct mutants or from hybridization involving desirable mutants [2]. In India alone, more than 300 mutant cultivars belonging to more than 55 plant species have been developed / released for cultivation [3]. Among the two kinds of mutants, micromutants with small changes in characters of the parental genotype might be more useful than macromutants because of their easy adoption to the agro-ecological and environmental condition of the parental genotype [4]. Successful use of micromutations as a method of crop improvement requires information on efficiency of mutagenic treatment in inducing micromutations, direction and magnitude of induced variation in the traits. It also requires an effective and efficient methodology for identification of desirable micromutants. The "efficacy" of mutagenic treatments can be assessed by their potential to produce more superior mutations [5]. With the above points in view, a mutation breeding project in finger millet (*Eleusine coracana* Gaertn.) was undertaken using three mutagens.

Materials and methods

The material for this study comprised of two morphologically distinct varieties of finger millet i.e., VR 708 (short height, early maturing with brown seeds) and GPU 26 (tall, late maturing with light brown seeds). Well filled seeds of the two varieties were treated with three doses each of a physical mutagen - gamma rays and two chemical mutagens - ethyl methane sulphonate (EMS) and nitroso guanidine (NG) employed separately and in combinations. The nine single mutagenic treatments were 150, 300 and 450 Gy of gamma rays, 0.15, 0.30 and 0.45% of EMS and 0.015, 0.030 and 0.045% of NG coded as G1, G2, G3, E1, E2, E3, N1, N2 and N3, respectively. The two combination treatments were 300 Gy gamma rays + 0.30% EMS and 300 Gy gamma rays + 0.030% NG, coded as GE2 and GN2, respectively. Dry seeds were irradiated with gamma rays (^{60}Co) at BARC, Trombay. For treatment with EMS and NG, the seeds were pre-soaked in distilled water for 10 hours, blotted dry and then treated with their freshly prepared aqueous solutions for 6 hours with intermittent shaking. For combination treatments, seeds were first irradiated with 300 Gy gamma rays and then treated with 0.30% EMS or 0.030% NG solution in the same manner as described above. After treatment, the seeds were thoroughly washed with running water to

leach out the residual chemicals and then dried on blotting paper.

The M_1 generation was grown and harvested as treatment bulk. In M_2 generation, chlorophyll and morphological macromutants were identified and harvested separately. For study of induction of micromutations, observations on 90 randomly selected normal looking plants from each treatment were taken and 165 M_2 plants (15 from each of 11 mutagenic treatments) were selected on the basis of higher yield. In M_3 generation, 165 M_2 plant progenies along with the parent variety were raised during *kharif* 2003 at EB-II section, Orissa University of Agriculture and Technology, Bhubaneswar. Separate trials, one for VR 708 and the other for GPU 26 were conducted in Compact Family Block Design with three replications putting treatments in main plot and selected plant progenies in sub-plot [6]. The seedlings of each plant progeny were transplanted in one row of 2.5 m length with a spacing of 30 × 10 cm. Ten random normal looking plants per progeny in each replication were harvested and data on mean yield/plant were analyzed. From each treatment four of the fifteen progenies (26.7% selection intensity) were selected on the basis of higher yield and within progeny selection was done at 30% selection intensity. In all, 44 selected mutant progenies from the eleven mutagenic treatments were carried to the next generation. In M_4 generation, separate trials with 44 mutant progenies and respective parental variety were conducted during *kharif* 2004 in randomized block design with three replications. Each progeny was grown in a plot of 3 rows of 3m length with a spacing of 22.5 × 10 cm. Observations on days to flowering and maturity were taken on plot basis and other seven biometric characters were recorded on ten random plants per progeny in each replication. The mean data were used for statistical analysis following standard procedures [7].

Results and discussion

The analysis of variance for yield/plant in M_3 generation showed highly significant differences among the treatment means in both the varieties. The yield/plant ranged from 6.380g (NI) to 6.993g (E2) in VR 708 and 8.468g (GN2) to 9.225g (E2) in GPU 26 in different treatments (Table 1). The narrow range of yield among the treatments seems apparent, since only the high yielding M_2 progenies were advanced to M_3 generation resulting in convergence of the variation. However, the results indicated the possibility of exercising even further selection in the treatments to identify relatively more superior individuals. A comparison of the yield of mutagenic treatments with that of the parent revealed that nine of the eleven treatments were significantly superior in both the varieties and hence, these treatments can be expected to produce desirable progenies in the next generation. This was substantiated in the production of high yielding progenies in M_4 generation.

Significant variation for yield among the 165 M_3 progenies of each treatment was also observed in both the varieties (Table 1). This is in agreement with the earlier reports in finger millet [8] and cowpea [9]. The yield/plant of the 165 progenies ranged from 5.61g to 8.73g in VR 708 and 7.25g to 12.10g in GPU 26, while in the parental varieties it was 6.347 and 8.383g, respectively. Further, the range of yield variation of the progenies within treatments indicated that the induced variation in yield in both the varieties was wide and in both directions in comparison to the parental varieties, offering scope for further selection. This was evident from the selection response of M_4 generation. Although majority of the progenies produced higher yield than the parent, only 61 progenies in VR 708 and 65 in GPU 26 were significant. The M_3 progenies, which produced significantly higher yield than the parental varieties were considered as superior to advance further. In both the varieties, mutagenic treatments produced superior progenies of variable number. More number (10) of superior progenies were obtained in treatments E2 of VR 708 followed by G3 with 7 and G2, N2, N3, GE2 with 6 each and GI, E3, GN2 with 5 each. In case of GPU 26, the treatments E2, E3 and N2 produced superior progenies of 9 each, closely followed by N3 (8), G2 (7) and GE2 (6). Of the 61 superior progenies of VR 708, 18 were each obtained from gamma rays and EMS treatments followed by 14 from NG and 11 from combination treatments. Similarly, out of the 65 superior progenies of GPU 26, the highest of 20 were each obtained from EMS and NG treatments, while 17 were obtained from gamma rays and only 8 from the two combination treatments.

The usefulness of any mutagenic agent in crop improvement depends not only on its ability to induce high frequency of mutations, but also on its capacity to induce large proportion of desirable changes. The "efficacy" of a mutagenic treatment should be assessed by its potential to produce more of useful mutations in different traits [5]. In this study, the character changes envisaged for improvement of productivity of finger millet were early flowering and maturity, short plant height and increase in tillers/plant, fingers/ear, finger length, 1000-grain weight, ear weight/plant and yield/plant. Evaluation of the 44 selected mutant progenies in M_4 generation indicated that most of the progenies differed significantly from the parent variety in one or more traits in both positive as well as negative direction. The mutant progenies showing significant changes in the desired direction from the parent variety in any of the above-mentioned traits were classified as superior or useful mutant for that trait.

Table 1. Mean yield (g/plant) and between progeny variation in different mutagenic treatments in M_3 generation

Treatment	Tr. code	Treatment mean yield	Between progeny variation		Superior progenies	
			Yield range	F-value	Progenies/ treatment	Progenies/ mutagen
Variety VR 708						
Gamma rays						
150Gy	G1	6.624*	5.83-8.05	18.68*	5	
300 Gy	G2	6.765*	5.82-8.34	28.98*	6	18
450 Gy	G3	6.829*	5.74-8.72	6.72*	7	
EMS						
0.15%	E1	6.448	5.71-7.51	11.78*	3	
0.30%	E2	6.993*	5.62-8.61	56.03*	10	18
0.45%	E3	6.734*	5.61-8.73	65.68*	5	
NG						
0.015%	N1	6.380	5.72-7.31	13.97*	2	
0.030%	N2	6.572*	5.85-7.42	11.59*	6	14
0.045%	N3	6.740*	5.61-8.52	22.48*	6	
Combinations						
300 Gy gamma rays + 0.30% EMS	GE2	6.606*	5.73-8.39	17.26*	6	11
300 Gy gamma rays + 0.030% NG	GN2	6.656*	5.71-8.41	36.12*	5	
	Parent	6.347				
	CD(5%)	0.135				
Variety GPU 26						
Gamma rays						
150Gy	G1	8.629*	7.90-9.74	9.08*	5	
300 Gy	G2	8.866*	7.65-10.90	25.04*	7	17
450 Gy	G3	8.636*	7.25-10.01	13.97*	5	
EMS						
0.15%	E1	8.563	7.65-10.64	8.79*	2	
0.30%	E2	9.225*	7.62-11.18	23.82*	9	20
0.45%	E3	9.168*	7.53-12.10	70.97*	9	
NG						
0.015%	N1	8.749*	7.65-11.04	21.42*	3	
0.030%	N2	9.188*	7.40-11.23	107.65*	9	20
0.045%	N3	8.937*	7.52-10.46	89.94	8	
Combinations						
300 Gy gamma rays + 0.30% EMS	GE2	8.794*	7.71-11.24	71.96*	6	8
300 Gy gamma rays + 0.030% NG	GN2	8.468	7.37-10.50	6.71*	2	
	Parent	8.383				
	CD(5%)	0.195				

*In treatment mean yield indicates significant increase over parent variety
*In F-value indicates significant difference between progenies within treatment

In the present study, out of the 44 mutant progenies evaluated in each set, 42 in VR 708 and 36 in GPU 26 exhibited superiority over their respective parents in one or more traits. Of these, 36 (85.71%) progenies of VR 708 and 25 (69.44%) of GPU 26 were superior in multiple traits. Among them, the highest proportion was obtained from EMS treatments followed by NG treatments in both the varieties, indicating their superiority over others for induction of useful mutants in multiple traits.

The frequency of superior mutants (Table 2) having desirable changes in nine traits in the set of mutants of VR 708 was the highest in the treatment GE2 (14.05%) followed by E3 (12.40%) and N3 (11.57%). In GPU 26, such mutants were obtained in higher frequency in E2 (18.48%) followed by N2 (13.04%) and E3 (10.87%) while it was very low in N3 (3.26%) and GE2 (4.35%). Similar differential efficacy of mutagenic treatments has also been reported earlier in finger millet [8] and green gram [10-12]. Considering the mutagen-wise distribution of superior mutants, it was observed that in VR 708, the frequency of superior mutants was higher in EMS treatments (28.93%) closely

Table 2. Distribution of progenies with superior mutation for different characters in M_4 generation

Tr. No.	Tr. code	Days to flowering	Days to maturity	Plant height	Tillers/ plant	Fingers/ ear	Finger length	1000-grain weight	Ear weight/ plant	Yield/ plant	Total	%
Variety VR 708												
1	G1	-	-	1	2	3	2	2	-	-	10	8.26
2	G2	1	1	-	-	3	2	1	1	-	9	7.44
3	G3	-	-	1	-	3	4	1	1	1	11	9.09
4	E1	2	1	-	-	1	2	2	-	-	8	6.61
5	E2	1	-	-	-	2	1	3	3	2	12	9.92
6	E3	3	2	1	-	1	1	4	1	2	15	12.40
7	N1	3	1	-	-	-	-	4	-	-	8	6.61
8	N2	1	2	-	-	-	1	2	-	-	6	4.96
9	N3	2	2	1	1	1	3	3	-	1	14	11.57
10	GE2	3	3	1	-	2	3	4	-	1	17	14.05
11	GN2	-	1	-	1	3	3	1	1	1	11	9.09
	Total	16	13	5	4	19	22	27	7	8	121	
	%	13.22	10.74	4.13	3.31	15.70	18.18	22.31	5.79	6.61		
Mutagens												
Gamma rays		1	1	2	2	9	8	4	2	1	30	24.79
EMS		6	3	1	-	4	4	9	4	4	35	28.93
NG		6	5	1	1	1	4	9	-	1	28	23.14
Combinations		3	4	1	1	5	6	5	1	2	28	23.14
Variety GPU 26												
1	G1	-	-	-	-	1	3	1	-	-	5	5.43
2	G2	1	1	1	-	2	1	-	1	1	8	8.70
3	G3	-	-	1	-	2	2	1	-	-	6	6.52
4	E1	-	1	1	1	1	3	-	1	1	9	9.78
5	E2	2	2	2	3	3	-	1	2	2	17	18.48
6	E3	1	1	1	2	2	-	-	2	1	10	10.87
7	N1	-	-	1	-	3	1	1	2	1	9	9.78
8	N2	-	-	1	2	3	2	-	2	2	12	13.04
9	N3	-	-	-	-	1	1	-	1	-	3	3.26
10	GE2	-	-	-	-	1	1	-	1	-	4	4.35
11	GN2	1	-	-	2	3	2	-	1	-	9	9.78
	Total	5	5	8	10	22	16	4	13	9	92	
	%	5.43	5.43	8.70	10.87	23.91	17.39	4.35	14.13	9.78		
Mutagens												
Gamma rays		1	1	2	-	5	6	2	1	1	19	20.65
EMS		3	4	4	6	6	3	1	5	4	36	39.13
NG		-	-	2	2	7	4	1	5	3	24	26.09
Combinations		1	-	-	2	4	3	-	2	1	13	14.13

followed by gamma rays (24.79%), as against 23.14% each in NG and combination treatments. In GPU 26, the frequency was the maximum (39.13%) in EMS treatments followed by NG (26.09%) and gamma rays treatments (20.65%). The EMS treatments in VR 708 produced high frequency of superior mutants in four traits, viz. days to flowering, 1000-grain weight, ear weight/plant and yield/plant, as against six traits in the variety GPU 26. Similar high efficacy of EMS treatments have also been reported earlier [8, 10, 12]. However, in both the varieties, gamma rays induced higher frequency of superior mutants for finger length while EMS induced more superior mutants for ear weight/plant and yield/plant. A comparative study of frequency of superior mutants for different traits revealed that in case of VR 708, the maximum frequency of superior mutants was recorded for 1000-grain weight (22.31%) followed by finger length (18.18%) and fingers/ear (15.70%) while in case of GPU 26, the maximum superior mutants were in fingers/ear (23.91%) followed by finger length (17.39%). This indicated that mutagenic treatments were effective in inducing more positive changes in these traits. Similar results exhibiting genotypic differences in the frequency of superior

mutations in different traits were also reported in finger millet [8] and green gram [10-12].

In M_4 generation, out of the 44 mutant progenies of each variety, 8 in VR 708 and 9 in GPU 26 produced significantly higher yield than their respective parent variety. These productive mutants showed diverse changes in characters such as, days to flowering, days to maturity, height and other direct yield components like tiller number, fingers/ear, finger length and 1000-grain weight (Table 3). Out of the 8 M_4 productive mutants of VR 708, four were from EMS; two were from combination and one each from gamma rays and NG treatments. Similarly, of the 9 productive mutants of GPU 26, four were from EMS treatments followed by three from NG, one each from gamma rays and combination treatments. In both the varieties, EMS treatments appeared to be more effective in producing high yielding mutants.

The efficacy of mutagenic treatments in inducing high yielding mutants could be assessed in M_3 and M_4 generations [12, 13]. Simultaneous consideration of production of high yielding progenies in both M_3 and M_4 generations in both varieties revealed EMS to be more effective, as it produced more number of desirable progenies followed by NG and gamma rays. Similar results suggesting higher effectiveness of the alkylating agents were also reported earlier in finger millet [8], lentil [14], mungbean [15] and blackgram [13]. Higher effectiveness of the alkylating agents 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 [16] described the mutagens belonging to the nitroso group as "super mutagens" in view of their greater mutagenic effects, a consequence of their alkylating ability on the gene directly. It has also been reported earlier that selection is more effective in both M_2 and M_3 generations [12, 17, 18]. This inference was further substantiated in the present study.

Table 3. Character changes observed in the high yielding mutant progenies of VR 708 and GPU 26 in M_4 generation

Sl.No.	Name of the progeny	Yield/plant (g)	Significant changes in characters from respective parent variety
Variety VR 708			
1	VG3-3	9.76	Late flowering and maturity and increase in plant height, fingers/ear and finger length
2	VE2-2	9.31	Early flowering, increase in height and 1000-grain weight
3	VE2-4	9.68	Late flowering and maturity and increase in plant height, fingers/ear and finger length
4	VE3-1	9.29	Early flowering and maturity and increase in 1000-grain weight
5	VE3-3	9.74	Increase in fingers/ear and 1000-grain weight
6	VN3-1	9.53	Early flowering and maturity and increase in plant height, tillers/plant, finger length and 1000-grain weight
7	VGE2-4	9.33	Increase in plant height, fingers/ear, finger length and 1000-grain weight
8	VGN2-1	9.46	Increase in height,tillers/plant, fingers/ear, finger length and 1000-grain weight
	Parent (VR708)	7.62	
Variety GPU 26			
1	GG2-1	12.73	Early flowering and maturity, short height and increase in fingers/ear
2	GE1-2	12.86	Late flowering and maturity and increase in tillers/plant and finger length
3	GE2-2	12.77	Increase in tillers/plant and fingers/ear
4	GE2-4	13.20	Late flowering
5	GE3-4	14.23	Late flowering and maturity and increase in tillers/plant and fingers/ear
6	GN1-2	12.61	Increase in height, finger length and 1000-grain weight
7	GN2-1	13.27	Late maturity and increase in tillers/plant and fingers/ear
8	GN2-2	12.65	Short plant height and increase in tillers/plant, fingers/ear and finger length
9	GGE2-3	13.37	Late flowering and maturity and increase in finger length
	Parent (GPU26)	10.21	

Considering treatment-wise distribution of high yielding mutant progenies in M_3 and M_4 generations in both the varieties, it was evident that the mutagenic treatment E2 to be most effective as it induced the highest number (19 in M_3 and 4 in M_4) of progenies followed by E3, N2 and GE2 treatments. Hence, it may be inferred that the treatments 0.30% and 0.45% EMS, 0.030% NG and combination treatment of 300 Gy gamma ray + 0.30% EMS could be more effective in inducing useful mutations in finger millet.

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