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Induced Mutations in Foxtail Millet (*Setaria italica* Beauv.)
II. Viable Mutations in Ear Characters Induced by Gamma Rays, EMS and dES

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Summary. Viable mutations for ear characters in two cultures of foxtail millet (*Setaria italica* Beauv.) were induced by individual and combined treatments of gamma rays, EMS (ethyl methanesulphonate), and dES (diethylsulphate). Four doses of gamma rays (10Kr to 40Kr), and four durations (6hrs to 24hrs) each of EMS(0.1 %) and dES(0.1 %) were used. In the combined treatments, only two durations (6hrs, 12hrs) of the chemical mutagens were used following each of the four doses of gamma rays.

The mutation frequencies were recorded as mutants per 100 M_2 plants and these were found to be higher in MU-1 than in MU-2 in all the treatments except dES. The frequencies also increased with increase in dose or duration of treatments and were found to be much higher in the combined treatments than in the individual treatments. In several combined treatments, a synergistic effect was observed in culture MU-1, the degree of synergism ranging from 1.01 to 1.62. Thus there was a differential response by the two cultures to the mutagenic treatments. The mutation spectrum was also found to be wider in culture MU-1, where 11 different kinds of mutants were recorded compared with only eight kinds of mutants recorded in MU-2.

Introduction

In an earlier communication (Gupta and Yashvir 1975), the frequencies and spectrum of chlorophyll mutations, induced by individual and combined treatments of gamma rays, EMS and dES, in two cultures (MU-1, MU-2) of foxtail millet (*Setaria italica* Beauv.) were reported and discussed in some detail. The number of sectors per spike and the efficiency and effectiveness of mutagenic treatments were also worked out. The specificity of the mutagens for different kinds of chlorophyll mutation was also discussed. The present paper describes the frequencies and spectrum of viable mutations for ear characters in the two cultures of foxtail millet.

Material and Methods

Commercial seed of foxtail millet was selfed for several generations and two distinct cultures were isolated. These two cultures were: (a) a bristled culture having a moisture content of 9.6 % and designated as MU-1; and (b) a nonbristled culture having a moisture content of 10.8 % and designated as MU-2. The moisture content was determined by drying a portion of seed in an incubator. Both the cultures were subjected to similar treatments by gamma rays, EMS and dES. The treatments included the following:

(i) Irradiation of fresh seed at the following doses: 10, 20, 30 and 40Kr.
(ii) Soaking of fresh seed with 0.1 % aqueous solution of EMS for the following durations: 6, 12, 18 and 24 hrs.
(iii) Soaking of fresh seed with 0.1 % aqueous solution of dES for the following durations: 6, 12, 18, 24 hrs.
(iv) Seed irradiated with 10, 20, 30 and 40 Kr and soaked in 0.1 % aqueous solution of EMS for 6 and 12 hrs.
(v) Seed irradiated with 10, 20, 30, 40 Kr and soaked in 0.1 % aqueous solution of dES for 6 and 12 hrs.

The gamma rays were secured from gamma cell-200 having a 2000 curie Co^{60} source, located at the Division of Genetics, Indian Agricultural Institute, New Delhi. EMS from Eastman Kodak Chemicals and dES of BDH Chemicals Ltd were used for the preparations of aqueous solutions of chemical mutagens. Treatments with chemical mutagens were given with intermittent shaking. In each treatment, 200 seeds were treated and grown in randomized block design with four replications. Seeds were initially sown in a nursery and the surviving seedlings were transplanted on the 25th day. In the plots, the rows were placed two feet apart and the distance between plants in a row was ten inches.

The seed from each spike of a plant in M_1 generation was harvested separately and the first two spikes from each plant were used for raising the M_2 generation. It was not possible to study segregation for viable mutations in all spike progenies, so small samples were taken from each treatment for the study of viable mutations. No less than 50 M_2 families were raised in a treatment.

The behaviour of M_2 mutants was studied in M_3 generation by testing them in progeny rows.

Table 1. Frequencies of viable mutations for ear characters, recorded as mu-

Treatment	Dose or duration	Mutants			
		Sterile tip	Sterile base	Gappy ear	Compact ear
Gamma rays	10Kr	0.18	-	0.91	0.67
	20Kr	0.84	-	0.71	0.65
	30Kr	0.24	0.42	0.42	0.66
	40Kr	0.31	0.93	3.85	0.53
EMS(0.1%)	6hrs	0.96	-	1.09	0.51
	12hrs	0.31	-	1.01	0.42
	18hrs	0.55	-	2.21	-
	24hrs	0.18	-	1.89	1.35
dES(0.1%)	6hrs	-	-	0.69	-
	12hrs	0.19	-	0.51	-
	18hrs	-	-	1.23	-
	24hrs	0.12	-	1.40	-
Gamma rays (10Kr) EMS(0.1%)	6hrs	0.54	-	1.23	-
	12hrs	1.11	-	1.84	0.43
Gamma rays (20Kr) EMS(0.1%)	6hrs	0.77	-	1.31	1.88
	12hrs	1.24	-	1.47	1.09
Gamma rays (30Kr) EMS(0.1%)	6hrs	0.31	0.83	0.80	0.94
	12hrs	1.65	-	3.40	0.33
Gamma rays (40Kr) EMS(0.1%)	6hrs	-	-	2.47	1.89
	12hrs	2.26	-	2.88	-
Gamma rays (10Kr) dES(0.1%)	6hrs	0.91	-	1.38	-
	12hrs	-	0.37	1.29	0.57
Gamma rays (20Kr) dES(0.1%)	6hrs	-	0.34	2.25	0.54
	12hrs	-	0.63	2.76	0.23
Gamma rays (30Kr) dES(0.1%)	6hrs	1.55	-	2.76	-
	12hrs	1.22	-	4.04	-
Gamma rays (40Kr) dES(0.1%)	6hrs	-	1.14	4.07	-
	12hrs	0.83	2.06	6.22	-

Results

The mutants in M_2 generation were identified on the basis of segregation in progeny rows. These mutants included a large number of plants which carried mutations for more than one character. Mutations for ear characters were found associated with mutants for a variety of other morphological characters involving structures like leaf, culm and tiller. Frequencies of mutants for ear characters and their spectrum will be dealt with irrespective of whether they were present as single mutants or were found associated with other mutant characters.

1. Frequencies of Viable Ear Mutants

As recommended by Gaul (1964), the frequencies of mutations were recorded as mutants per 100 M_2 plants. The frequencies of 11 different kinds of ear mutants recorded in culture MU-1 are presented in Table 1. In the other culture, MU-2, only eight different kinds of ear mutants were recorded and their frequencies are presented in Table 2.

2. Spectrum of Ear Mutations

The ear mutants included the following:

(1) Compact ears: Fig.1. These mutants were recorded only in culture MU-1.

(2) Branched ears: Fig.2. These mutants were also available in culture MU-2.

(3) Sorghum type ears: Figs.3,4. In certain mutants, the spike became loose and had a bigger diameter giving it the shape of a *Sorghum* type ear.

tants per 100 M_2 plants, in culture MU-1 of foxtail millet *(Setaria italica)*

Sorghum ear	Branched ear	Long ear	Short ear	Short bristles	Purple lemma	Coiled neck	Total	Degree of synergism
-	-	-	-	1.34	-	-	3.10	
-	0.32	-	-	2.79	0.26	0.26	5.83	
0.36	0.18	-	0.23	0.16	-	0.36	5.10	
-	0.42	-	-	5.22	-	-	7.86	
0.13	0.19	-	-	1.19	0.32	0.13	4.52	
0.12	0.12	0.24	0.23	1.02	-	0.30	3.84	
0.18	-	0.23	-	2.34	-	0.12	5.70	
0.30	0.48	0.18	0.18	1.83	-	-	6.39	
-	-	-	-	0.64	-	-	1.33	
-	-	-	-	1.28	-	-	1.98	
0.12	-	0.12	0.86	0.74	-	-	3.07	
-	-	-	-	2.72	0.17	-	4.41	
0.20	0.61	-	-	0.89	-	0.34	3.81	
0.43	-	0.24	-	2.19	-	0.18	6.42	
0.23	-	-	-	0.62	-	0.62	5.43	
-	0.62	-	0.23	2.68	-	-	7.43	
0.31	0.21	0.21	-	1.83	-	0.28	5.74	
0.64	0.57	0.80	-	2.09	-	-	9.48	
1.58	-	0.62	-	2.98	-	0.31	9.85	
-	1.58	-	1.59	6.34	2.26	-	16.91	1.45
-	0.16	-	-	1.87	0.76	-	5.08	1.15
0.37	-	0.61	-	2.96	-	-	6.17	1.22
-	-	-	-	1.50	-	0.54	5.17	
0.55	-	-	0.31	3.02	0.39	-	7.89	1.01
-	-	-	-	3.67	-	-	7.98	1.24
-	0.62	1.31	-	4.13	-	-	11.32	1.60
0.32	-	-	0.97	4.93	-	1.28	12.71	1.38
-	-	1.45	-	5.39	-	-	15.95	1.62

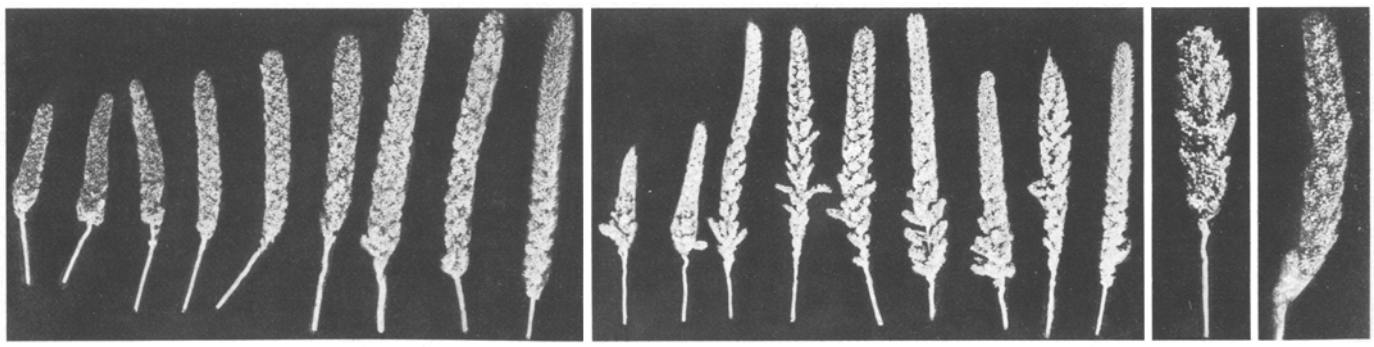

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Fig.1-4. Viable mutations for ear characters in foxtail millet recorded in M_2 generation. Fig.1: Variability in the compactness of ear in culture MU-1 (control on extreme right). Fig.2: Variability in the morphology of branched ears (control on extreme right). Fig.3: A *Sorghum* type ear with coiled neck. Fig.4: Another *Sorghum* type ear with coiled neck

Table 2. Frequencies of viable mutations for ear characters, recorded as mutants per 100 M_2 plants, in culture MU-2 of foxtail millet *(Setaria italica)*

Treatment	Dose or duration	Mutants								
		Sterile tip	Sterile base	Gappy ear	Lax ear	Long ear	Short ear	Coiled neck	Purple lemma	Total
Gamma rays	10Kr	0.13	1.65	0.89	0.58	-	0.13	-	-	3.38
	20Kr	0.84	1.93	1.22	-	-	-	0.26	-	4.25
	30Kr	1.47	2.08	1.94	-	-	-	-	-	5.49
	40Kr	0.99	3.94	1.71	0.31	-	-	0.15	-	7.10
EMS (0.1%)	6hrs	0.26	2.54	1.67	0.38	-	-	-	0.13	4.98
	12hrs	0.54	2.13	1.02	0.54	-	-	-	-	4.23
	18hrs	0.45	2.65	1.35	0.40	-	-	-	-	4.85
	24hrs	0.14	2.19	1.34	0.35	-	0.20	0.21	-	4.43
dES (0.1%)	6hrs	0.48	2.35	0.65	0.35	0.18	-	-	-	4.01
	12hrs	0.51	2.67	0.62	0.25	-	0.26	-	0.44	4.75
	18hrs	-	2.84	1.68	0.13	-	-	-	-	4.65
	24hrs	0.73	2.63	0.99	-	0.34	-	-	-	4.69
Gamma rays (10Kr) EMS(0.1%)	6hrs	-	2.39	1.25	0.29	-	-	-	-	3.93
	12hrs	0.43	4.36	0.78	0.52	-	-	-	-	6.09
Gamma rays (20Kr) EMS(0.1%)	6hrs	-	3.02	0.76	0.25	-	-	-	-	4.03
	12hrs	0.16	2.00	1.52	0.56	-	-	-	-	4.24
Gamma rays (30Kr) EMS(0.1%)	6hrs	-	2.68	0.67	-	-	-	-	0.29	3.64
	12hrs	-	3.28	3.69	-	-	-	-	-	6.97
Gamma rays (40Kr) EMS(0.1%)	6hrs	0.73	2.32	1.36	-	0.29	-	-	-	4.70
	12hrs	1.34	3.43	3.82	-	-	-	0.57	-	9.16
Gamma rays (10Kr) dES(0.1%)	6hrs	0.25	2.25	0.63	0.32	-	-	-	-	3.45
	12hrs	-	2.52	0.77	0.21	-	-	-	-	3.50
Gamma rays (20Kr) dES(0.1%)	6hrs	0.60	2.04	1.14	0.51	-	-	-	0.42	4.71
	12hrs	-	2.74	1.27	0.58	-	-	-	-	4.59
Gamma rays (30Kr) dES(0.1%)	6hrs	1.15	6.03	0.66	1.32	-	-	-	-	9.16
	12hrs	0.88	5.70	1.65	0.77	-	-	-	-	9.00
Gamma rays (40Kr) dES(0.1%)	6hrs	1.03	3.11	3.25	1.03	0.44	-	-	-	8.86
	12hrs	-	4.61	1.58	1.05	-	-	0.79	1.05	9.08

(4) Ears with sterile tip: Fig.5. This mutant ear character was often found associated with other mutant ear characters such as gappy ear.

(5) Ears with sterile base: Figs.5,6,10. This mutant was more frequent in culture MU-2 than in MU-1 and was often found associated with gappy ears. The morphology of the ears with sterile base also showed considerable variability.

(6) Gappy ears: Fig.5. Gappy ears had distinct gaps along the length of the ears. This character was found associated with other characters such as sterile tip and sterile base.

(7) Long ears: Figs.6,7. While the mean length of the spike in the control was 10.6 cms and 10.1 cms in the two cultures, with a maximum of 16.3 cms, the long ear mutants had spikes as long as 30 cms.

(8) Short ears: Fig.8. The smallest ears in the control measured 7.2 cms and 7.4 cms for the two cultures. In short ear mutants, the length of the ear varied from 2.0-4.0 cms.

(9) Coiled neck: Fig.9. The degree of coiling in the neck varied in the different cases. These mutants were rather infrequent in comparison with other mutants.

(10) Short bristles: Fig.11. In bristled culture MU-1, mutations for short bristles were also recorded. The short bristles could be present all along the length of the ear or could be restricted to the base of the ear.

Discussion

The mutation frequencies in graminaceous crops are generally recorded by any one of the following three methods: (a) mutations per 100 M_1 plants; (b) mutations per 100 M_1 spikes; and (c) mutants per 100 M_2

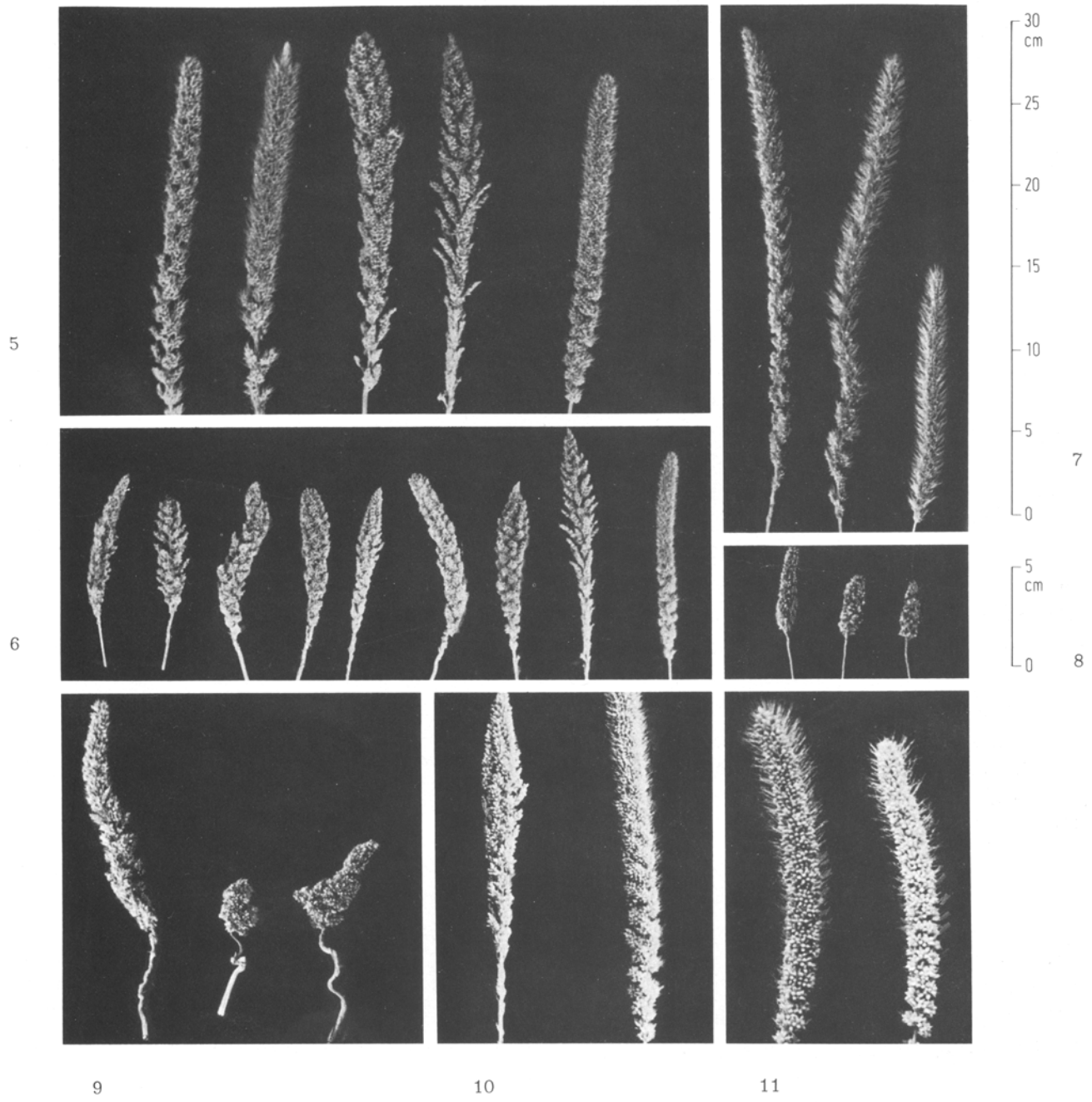

Fig.5-11. Viable mutations for ear characters in foxtail millet recorded in M_2 generation (contd.). Fig.5: Four different mutants showing gappy ear; gappy ear with sterile tip; gappy ear with short bristle and gappy ear with sterile base (control on extreme right). Fig.6: Variability in the morphology of ears with sterile base (control on extreme right). Fig.7: Long ear mutants with a control. Fig.8: Short ear mutants. Fig.9: Mutant ears with coiled neck. Fig.10: A mutant ear showing sterile base and a control ear. Fig.11: A mutant ear showing short bristles at base and a control ear

plants. Gaul (1964) argued that methods (a) and (b) would give a relative underestimate of mutation frequencies at higher doses. He recommended that mutation frequencies should be recorded as per 100 M_2 plants. Based on this, the frequencies of viable mutations during the present study were recorded by the method (c) outlined above. However, the present authors (Gupta and Yashvir 1975) had earlier shown in the present material, using chlorophyll mutations, that all three methods give equally reliable estimates of

mutation frequency. The only factor which we thought could contribute to a biased estimate is that only the first two spikes of each M_1 plant were taken for raising the M_2 generation. There are, however, some reports (see Gupta and Yashvir 1975) available in the literature which indicate that the mutation frequencies could be higher in the late tillers.

The mutation frequencies recorded as mutants per 100 M_2 plants in the present study can, however, be expressed in terms of mutation frequencies of the embryo germ track cells. Gaul (1964) gave the following relationship for this purpose: $u = m/f$, where u is the mutation frequency per germ track cell, m is the mutation frequency in terms of mutants per 100 M_2 plants and f is the constant which is the segregation ratio, assumed to be 20% in the case of barley. The segregation ratio is not very different in foxtail millet (Gupta and Yashvir 1975). Therefore, the mutation frequencies in terms of embryo germ track cells would be four to five times those recorded in Tables 1 and 2.

When the mutation frequencies are compared in two cultures of foxtail millet, it is obvious that the frequencies were higher in culture MU-1 than in MU-2 in all the treatments except individual dES treatments, where the situation was the reverse. There is a general trend toward increasing mutation frequency with the increase in dose or duration of the treatment. In general the mutation frequency was also higher in the combined treatments than in the corresponding individual treatments, so much so that in culture MU-1, in several combined treatments and particularly in gamma rays combined with dES, synergistic effects were observed. The degree of synergism in the different treatments was calculated following Sharma (1970) and the results are presented in the last column of Table 1. It is obvious that the synergism ranged from 1.01 to 1.62 in the different treatments. No synergistic effects were observed in culture MU-2. In fact, in some combined treatments, the mutation frequencies in culture MU-2 were lower than the corresponding individual treatments with the chemical mutagen. It is obvious that there was a considerable difference in response of the two cultures to different mutagenic treatments. This should, at least in part, be attributed to differences in the genetic make-up of the two cultures.

It is also obvious from Tables 1 and 2 that the mutation spectrum for mutations in ear characters is much wider in culture MU-1 than in culture MU-2. While in culture MU-1, 11 different types of ear mutants were obtained, in MU-2 only eight types were recovered. Variations were also observed in the frequencies of different ear mutations. In MU-1, the most frequent types were gappy ear and short bristles, while in MU-2 the most frequent was the sterile base and gappy ear. It is interesting to note that the sterile base, which is so frequent in MU-2, is very rare in MU-1. The question of the short bristles mutant would not arise in nonbristled culture MU-2.

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