

ETHYL METHANESULFONATE-INDUCED SEEDCOAT COLOR MUTANTS IN *Phaseolus vulgaris* L.

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ABSTRACT

Germinating seeds of a high yielding black bean (*Phaseolus vulgaris* L.) cultivar were treated with 0 (control), 0.0625, 0.125, 0.25 and 0.5% (v/v) buffered ethyl methanesulfonate (EMS) solutions with the purpose of exploring the possibility of inducing seedcoat color mutations. The highest frequency of mutations was induced by 0.25% EMS. The results indicate that EMS-induced genetic modifications can readily change the seedcoat color of the cultivar used to different hues of brown.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important source of low-cost protein in many countries, especially in South America. Its commercial acceptance, however, depends on various factors, one of which is the seedcoat color. Many times high-yielding cultivars with black seedcoat are not cultivated due to local preference for different seedcoat colors (Vieira, 1967; Moh, 1969, 1971, 1972; Guerra Chomon *et al.*, 1975; Tulmann Neto *et al.*, 1980).

The modification of the seedcoat color is usually accomplished by using the conventional backcrossing method. However, data obtained by several authors, among them Swarup and Gill (1968), Moh (1969, 1971, 1972), Guerra Chomon *et al.* (1975), Hussein and Disouki (1976), Saito *et al.* (1980) and Al-Rubeai (1982), have indicated

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that mutation induction is an efficient method for the modification of the seedcoat color, besides being much less time consuming. In some cases (Moh, 1969, 1971, 1972; Guerra Chomon *et al.*, 1975; Hussein and Disouki, 1976) the induced mutants had the same general characteristics as the original cultivar, including its yielding ability.

The present paper reports the results of an experiment designed to evaluate the possibility of changing the seedcoat color of a high-yielding bean cultivar by means of induced mutation.

MATERIAL AND METHODS

The black bean cv. Milionário 1732 (BAT 65), from the Centro Internacional de Agricultura Tropical (CIAT), Colombia, was used in this study. A total of 1,500 dry seeds (moisture content of approximately 14%) were placed in Petri dishes in a germinator at 28°C for a period of 24 hours. Individual groups of 300 seeds were transferred to 500 ml-Erlenmeyer flasks containing freshly prepared ethyl methanesulfonate (EMS) solutions of 0.0625, 0.125, 0.25 and 0.5% (v/v) concentration, respectively. EMS solutions were prepared in 0.02 M phosphate buffer, pH = 7.0. A control group of 300 seeds was treated only with the buffer solution. In each case the volume of the solution was about 2.5 times the seed volume. The Erlenmeyers were covered with aluminum paper and shaken on a "Burrell Wrist-Action" agitator for six hours at room temperature (ca. 24°C). Seeds were then washed in running tap water and immediately taken to the field for planting according to a randomized complete block design with six replications. Each plot consisted of two rows 5 m long and 0.60 m apart. In each row seeds were spaced 0.20 m.

Fifty M_2 seeds from each M_1 plant were planted in a single row. When an M_1 plant yielded less than 50 seeds all of them were planted. Spacing was the same as in the first generation. Each M_2 plant was harvested and threshed separately and M_3 seeds were scored for seedcoat color changes.

RESULTS AND DISCUSSION

As shown in Table I, the M_2 progeny of 11 M_1 plants derived from EMS-treated seeds segregated for seedcoat color. Mutation frequency was highest with 0.25% EMS, whether it was calculated on the basis of 100 treated seeds, 100 surviving M_1 plants or M_2 plants, which are methods commonly used. The value (2.33%) calculated on the basis of treated seeds corresponds to Walther's (1969) factor of effectiveness and indicates the number of independent mutational events that are induced by the mutagen. This calculation, however, provides an underestimation due to several factors acting singly or in combination. One of them is related to the chimeric structure of the M_1 plant (Lindgren *et al.*, 1970) resulting from the

Table I - Frequency of induced seedcoat color mutants in the M₂ generation.

EMS concentration (% v/v)	No. of treated seeds	No. of surviving M ₁ plants	M ₁ plants segregating for seed-coat color in M ₂			No. of M ₂ plants	M ₂ plants with mutant seed-coat color	
			No.	% of			No.	%
				treated seeds	surviving M ₁ plants			
0 (control)	300	265	0	0	0	ca. 2,000	0	0
0.0625	300	259	1	0.33	0.39	8,200	16	0.20
0.125	300	253	2	0.67	0.79	8,294	18	0.22
0.25	300	223	7	2.33	3.13	6,094	35	0.57
0.5	300	98	1	0.33	1.02	2,997	16	0.53

action of the mutagen on one or several initial cells. Another possibility is the occurrence of diplontic or haplontic selection (Gaul, 1960, 1961a) which may eliminate some of the cells carrying the mutation. It is also possible that segregation may be delayed until the M₃ generation (Gaul, 1961b; Gottschalk, 1961; Gottschalk and Wolff, 1983). All of these factors make it possible that the random seed sample used to raise the M₂ generation will not carry the induced mutation. It should also be considered that various phenotypically distinct seedcoat color mutants were found in the M₂ progeny of a single M₁ plant. Although allele tests have not been carried out as yet, at least some of these mutants may well be the result of different mutational events as judged by the variety of seedcoat colors produced and/or by differences in seed size. This may also contribute to an underestimation of mutation frequency.

The mutation frequencies estimated as percentage of surviving M₁ plants or as percentage of M₂ plants, the latter being the method favored by Gaul (1960), may be misleading. At higher concentrations of the mutagen there is an increase in sterility and lethality which reduces the number of plants, resulting in an apparent increase in mutation rate. Besides, a single M₁ plant which bears a single mutation may yield several mutants in the M₂ generation. As is apparent from Table I, only one M₁ plant was found carrying a mutation induced by 0.5% EMS, while seven were found from seeds treated with 0.25% EMS. The percentage of mutants calculated on the basis of M₂ plants would indicate that 0.5% EMS was about as effective as 0.25% in inducing mutations. This is probably the reason why Gaul (1961b) recommends such high doses of radiation as to result in about 90% lethality of M₁ plants. The authors are of a different opinion, however, for the higher the dose or concentration of the mutagen the greater the probability of severely affecting the genetic material. More useful

mutants are more probably induced by low to medium concentrations of the mutagen, as suggested by Kawai (1969) and Yonezawa and Yamagata (1977).

Data on the number of M_2 plants bearing seedcoat mutations which were raised from a single M_1 plant are presented in Table II. In three cases (rows number 573, 582 and 742) the percentages of mutants were close to 25%. This has been interpreted as indicating that a single cell primordium which gave rise to the respective M_1 plant carried the mutation, so that the whole plant was heterozygous (Motto *et al.*, 1975; Gottschalk and Wolff, 1983). In the other cases there was either excess or deficiency of mutant M_2 plants. Excess of mutants in this case might be the outcome of simultaneous mutations in two complementary seedcoat color genes in the cell primordium which originated the M_1 plant. This would lead one to expect a segregation ratio of 9 normal to 7 mutants, as seems to be the case for rows number 22, 252 and 810. Deficiency of mutants in the M_2 generation is not uncommon and the chimeric status of M_1 plants, diplontic and haplontic selection and delayed segregation, as

Table II - Number of M_2 plants with mutant seedcoat colors derived from a single M_1 plant.

EMS concentration (% v/v)	Identification no. of row with M_2 plants	No. of M_2 plants	No. of M_2 plants with mutant seedcoat color	Seedcoat color
0 (control)	Several	Several	0	Black
0.0625	22	43	16	Different hues of beige
0.125	573	31	7	Different hues of beige
	582	47	11	Different hues of beige
0.25	630	37	1	Red brown
	669	26	1	Red brown
	676	10	1	Red brown
	681	33	3	One yellow brown and two red brown
	742	38	10	Different hues of beige and yellow brown. All with either black or dark brown stripes.
0.25	763	26	2	One beige and one yellow brown
	810	36	17	Different hues of beige, yellow brown, yellow and grayish yellow
	252	36	16	Different hues of beige and yellow brown. All with either black or dark brown stripes.

previously cited, are again possible causes. Additionally, reduced size of mutated sectors (Lindgren *et al.*, 1970; Motto *et al.*, 1975) due to delayed action of the mutagen might have the same effect. However, whatever the explanation may be, it should be taken with caution in view of the low number of plants grown in the M₂ generation.

Of interest was the finding of two independent cases of mutant seedcoats having different hues of brown background with either black or darker brown stripes. In the M₃ generation from bean seeds treated with X-rays, Swarup and Gill (1968) also found seedcoat mutants having either black or brown streaks. The induced striped-color pattern reported here parallels the various cases of variegation especially studied in maize by McClintock (1965 and references therein). This and further experimental data obtained by Barbosa (unpublished) lead the authors to suggest that the striped mutants are the consequences of the activation, here by EMS, of transposable element(s). Such elements may exist silently in the genome (Fedoroff *et al.*, 1983; McClintock, 1984; Doring and Starlinger, 1986) and be expressed as a response to adverse conditions to which cells are exposed, including radiation, viral infection, "poisons of various sorts", etc. (McClintock, 1984).

RESUMO

Sementes de um cultivar produtivo de feijão (*Phaseolus vulgaris* L.) de tegumento preto, em germinação, foram tratadas com soluções tamponadas de etil-metanossulfonato (EMS) nas concentrações de 0 (controle), 0,0625, 0,125, 0,25 e 0,5% (v/v). O objetivo foi avaliar a possibilidade de induzir mutações da cor do tegumento. A maior frequência de mutações foi induzida por EMS a 0,25%. Os resultados indicam que o EMS é agente mutagênico eficiente, capaz de modificar a cor do tegumento do cultivar utilizado, produzindo mutantes apresentando diferentes tonalidades de marrom.

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