Rev. Brasil. Genet. 11, 3, 699-705 (1988)
(Brazil. J. Genetics)

# ETHYL METHANESULFONATE-INDUCED MUTATIONS IN *Phaseolus vulgaris* L.

Hélio M. Barbosa[1], José Eustáquio de S. Carneiro[2] and Clibas Vieira[3]

## ABSTRACT

Germinating seeds of *Phaseolus vulgaris* L. were treated with 0 (control), 0.0625, 0.125, 0.25, and 0.5% ethyl methanesulfonate (EMS) solutions. Hypocotyl and flower color mutations were detected in the $M_2$ generation mostly after 0.25% EMS treatment. The relative success of selecting for seedcoat color mutants based on the correlation between hypocotyl color and seedcoat color is discussed. For each mutagen concentration the frequency of chlorophyll deficiency mutations of the types *albina*, *xantha* and *viridis* increased in that order. Several morphologic mutants were found. A few of these recovered from the anomaly during later ontogenetic stages and may turn out to be good markers for genetic studies.

## INTRODUCTION

The utilization of induced mutations for the improvement of crop plants has yielded several mutants which have been used directly as new cultivars (Gottschalk and Wolff, 1983). Besides, many of the induced mutants, mainly the defective types which are not usually found among plants of commonly grown varieties, may be useful as material for genetic studies. This is especially important for species such as the common bean for which not many genetic markers are known. In addition, its system of reproduction and short life cycle make it an ideal plant for mutation breeding experiments.

[1] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570 Viçosa, MG, Brasil. Send correspondence to H.M.B.

[2] EMBRAPA/CNPAF, 74000 Goiânia, GO, Brasil.

[3] Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570 Viçosa, MG, Brasil.

The objectives of the experiment described here were to explore the possibility of inducing useful mutants in a high-yielding bean cultivar and to determine the spectrum and frequency of the induced mutants.

## MATERIAL AND METHODS

The cultivar used was "Milionário 1732" (BAT 65), bred at the Centro Internacional de Agricultura Tropical (CIAT), Colombia. The cultivar has red hypocotyls, violet flowers and black seeds. Seed treatment with ethyl methanesulfonate solutions and the experimental procedure up to the planting of $M_2$ seeds were described by Barbosa *et al.* (1988). In the $M_2$ generation, data were collected on hypocotyl and flower color mutations. Chlorophyll mutations were scored during the early stages of plant development. The occurrence of morphologic mutants was recorded from emergence until flowering time. Data were also taken on the frequency of sterility and lethality among the morphologic deviates and on the frequency of morphologic deviates which recovered from the anomaly during later stages of development.

## RESULTS AND DISCUSSION

According to Lamprecht (1935), pigment synthesis in the hypocotyl, flower and seedcoat of the bean plant depends on the presence of the basic dominant genes $P$ and $T$. When both are present, hypocotyl and flower color is determined by the multiple allelic series $V$ (red hypocotyl and violet flowers), $v^{lae}$ (pink hypocotyl and flowers) and $v$ (pink or green hypocotyl and white flowers) with partial to complete dominance (Lamprecht, 1935; Prakken, 1972). This relationship between hypocotyl and flower color is not always found. While varieties with white flowers always have green hypocotyls colored-flower varieties may have either green or colored hypocotyls (Miyake *et al.*, cited by Kooiman, 1931; Prakken, 1934). The pink phenotype of the hypocotyl is not easily noticeable (Prakken, 1940). For this reason and due to extreme difficulties in detecting pink hypocotyls among a large number of $M_2$ plants (about 25,000) under field conditions, as was the case in this study, the data presented in Table I refer to mutations from red to green hypocotyl. Table I also shows the mutation frequencies from violet to either white or pink flowers. A total of seven $M_1$ plants segregated for flower color in the $M_2$ generation, five of which for white and two for pink flowers. In agreement with Prakken (1934) and others, it was noted that white-flowered segregants always had green hypocotyls. Also, cases were found in the $M_2$ generation of plants having violet flowers and green hypocotyls.

The seedcoat color of beans is an important character determining the acceptance of seeds by consumers (Vieira, 1967; Moh, 1969, 1971). After examining the relationship between hypocotyl color and seedcoat color of 271 varieties, Moh

(1971) and Moh and Alan (1971a) found that black bean varieties always had red hypocotyls, white bean varieties always had green hypocotyls and colored-seed varieties might have either red or green hypocotyls. This led them to propose a screening technique to facilitate mutation work aimed at changing the seedcoat color of black-seeded varieties which was later used by Moh and collaborators (Guerra Chomon *et al.*, 1975). According to this procedure, the $M_2$ seedlings with green or pink hypocotyls should be saved for further examination while all seedlings with red hypocotyls should be discarded. Data on seedcoat color mutations obtained for the present study were presented by Barbosa *et al.* (1988) who reported eleven independent mutational events leading to seedcoat color changes. If Moh's technique had been applied, the authors would have isolated only six (about 55%) independent mutations which correspond to the number of hypocotyl color mutations shown in Table I. Thus, Moh's screening procedure should be recommended only if the investigator is interested in white seedcoat mutants. Otherwise, many desirable red-hypocotyl seedlings carrying mutations for seedcoat color genes will be unwittingly discarded.

Table I - Frequency of $M_1$ plants yielding $M_2$ progenies segregating for hypocotyl and flower color.

| EMS concentration (% v/v) | No. of surviving $M_1$ plants | $M_1$ plants segregating in the $M_2$ generation for: Green hypocothyl | | | Flower color: White | | | Flower color: Light pink | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % of Treated seeds | % of Surviving $M_1$ plants | No. | % of Treated seeds | % of Surviving $M_1$ plants | No. | % of Treated seeds | % of Surviving $M_1$ plants |
| 0 (control) | 265 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0625 | 259 | 2 | 0.67 | 0.77 | 1 | 0.33 | 0.39 | 0 | 0 | 0 |
| 0.125 | 253 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.25 | 223 | 3 | 1.00 | 1.35 | 3 | 1.00 | 1.35 | 2 | 0.67 | 0.90 |
| 0.5 | 98 | 1 | 0.33 | 1.02 | 1 | 0.33 | 1.02 | 0 | 0 | 0 |

As shown in Table II, the mutation spectrum for chlorophyll deficiency was limited to *albina, xantha* and *viridis*. These were also the only types detected by Al-Rubeai (1982) in the $M_2$ generation after treating seeds of the same species with gamma rays. Also in agreement with Al-Rubeai's data was the finding that *viridis* was by far the most frequently induced chlorophyll deficiency while *albina* was the least frequent. This held true for all EMS concentrations. This agreement with Al-Rubeai's (1982) results was not expected since, according to Nilan (1972), there is a considerable amount of data indicating that the spectrum of chlorophyll deficiency mutations induced by alkylating agents is different from that induced by gamma rays. In addition, there are plenty of data showing variations in the proportions of different chlorophyll mutations in barley as well as in other species caused by differences in experimental conditions (Nilan and Konzak, 1961; Nilan, 1972). The frequency of chlorophyll mutations estimated as percent of treated seeds (Table II) increased with increasing EMS concentrations up to 0.25%. This was also the concentration which induced the highest frequency of seedcoat color mutations as reported by Barbosa *et al.* (1988) for the same cultivar. This does justice to the fact that the scoring of chlorophyll-deficiency mutations is the method most commonly used to assess the effects of mutagens (Hansel, 1968; Nilan and Vig, 1976; Gottschalk and Wolff, 1983). With 0.5% EMS, the percentage of chlorophyll mutations decreased when calculated on the basis of number of treated seeds, while it was highest when estimated as percent of surviving $M_1$ plants. This was obviously due to the drastic effects of 0.5% EMS which severely reduced the number of $M_1$ plants.

Table II - Frequency of $M_1$ plants yielding $M_2$ progenies segregating for chlorophyll mutations.

| EMS concentration (% v/v) | No. of surviving $M_1$ plants | Segregating $M_2$ progenies | | | Albina | | Xantha | | Viridis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % of Treated seeds | % of Surviving $M_1$ plants | No. | % of total | No. | % of total | No. | % of total |
| 0 (control) | 265 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0625 | 259 | 16 | 5.33 | 6.18 | 2 | 12.50 | 5 | 31.25 | 9 | 56.25 |
| 0.125 | 253 | 21 | 7.00 | 8.30 | 1 | 4.76 | 5 | 23.81 | 15 | 71.43 |
| 0.25 | 223 | 25 | 8.33 | 11.21 | 4 | 16.00 | 6 | 24.00 | 15 | 60.00 |
| 0.5 | 98 | 16 | 5.33 | 16.33 | 1 | 6.25 | 4 | 25.00 | 11 | 68.75 |

Many different morphologic deviates from type were detected, such as absence or malformation of flower structures, abnormal pods, short internodes (dwarfs), excess or absence of branches, and several leaf anomalies, such as three cotiledonar leaves, *unifoliolata, bi-foliolata,* wrinkled leaves, coarse leaves, narrow leaflets, etc. Mutants apparently similar to some of these have been described by several authors for the same species after treatment with X-rays (Genter and Brown, 1941; Swarup and Gill, 1968), gamma rays (Moh and Alan, 1964, 1970, 1971b, 1971c; Moh, 1968; Al-Rubeai, 1982) or EMS (Tara Mohan, 1979). Table III contains data on the frequency of morphologic mutations as well as on the occurrence of sterility and lethality among the morphologic mutants. When estimated as percent of treated seeds, the mutation frequency decreased with increasing EMS concentration. This was also the case for concentrations of up to 0.25% when calculation was made on the basis of surviving $M_1$ plants. This may be attributed to the higher cell lethality and more severe genetic damage caused by higher mutagen concentrations, which reduced the number of $M_1$ plants, thus preventing the scoring of morphologic aberrations which might otherwise have been detected. With 0.5% EMS there was a striking reduction in the number of $M_1$ plants so that almost one third (31.63%) of the surviving plants segregated in $M_2$ for some kind of morphologic mutation. In addition, about 58% of the $M_2$ plants with morphologic changes were sterile. According to Gaul (1977), induced chromosome aberrations may be the major cause of sterility. Data on the $M_1$ generation for the same material were reported by Carneiro *et al.* (1987) who found that increased EMS concentrations caused an increase in sterility, as judged by a reduction in the number of seeds per pod and by the occurrence of malformed pods. Similar results were reported by Hussein and Disouki (1976). As shown in Table III, percent lethality among morphologic mutants increased with increasing EMS concentration. It should be stated, however, that some of the morphologic mutants previously mentioned recovered from the anomaly at later ontogenetic stages. Their numbers were 6, 7, 6 and 1 for the $M_2$ progenies derived from the

Table III - Frequency of $M_1$ plants yielding $M_2$ progenies segregating for morphologic mutations and frequency of sterility and lethality among the mutants.

| EMS concentration (% v/v) | No. of surviving $M_1$ plants | Segregating $M_2$ progenies | | | % of morphologic mutants which were | |
|---|---|---|---|---|---|---|
| | | No. | % of Treated seeds | % of Surviving $M_1$ plants | Sterile | Lethal |
| 0 (control) | 265 | 0 | 0 | 0 | 0 | 0 |
| 0.0625 | 259 | 50 | 16.67 | 19.30 | 34.00 | 6.00 |
| 0.125 | 253 | 47 | 15.67 | 18.58 | 29.79 | 8.51 |
| 0.25 | 223 | 33 | 11.00 | 14.80 | 39.39 | 9.09 |
| 0.5 | 98 | 31 | 10.33 | 31.63 | 58.06 | 12.90 |

0.0625, 0.125, 0.25 and 0.5% EMS treatments, respectively. This, again, reflects the damaging effects of 0.5% EMS. If in succeeding generations, some of these mutants prove to be affected only during the early stages of development they may turn out to be valuable markers for genetic studies.

## RESUMO

Sementes de *Phaseolus vulgaris* L., em germinação, foram tratadas com soluções de etil metanossulfonato (EMS) nas concentrações 0 (controle), 0,0625, 0,125, 0,25 e 0,5%. Na geração $M_2$ foram detectadas mutações afetando a cor do hipocótilo e a cor das flores, a maioria após tratamento com 0,25% EMS. Discute-se a técnica de seleção de mutantes da cor do tegumento baseada na correlação entre cor do hipocótilo e cor do tegumento. Para cada concentração de EMS a freqüência de mutantes clorofilianos dos tipos *albina, xantha* e *viridis* aumentou nesta ordem. Vários mutantes morfológicos foram detectados, dos quais uns poucos recuperaram-se da anormalidade em estágios ontogenéticos posteriores, podendo vir a ser bons marcadores genéticos.

## REFERENCES

Al-Rubeai, M.A.F. (1982). Radiation-induced mutations in *Phaseolus vulgaris* L. *Rev. Brasil. Genet. 5*: 503-515.

Barbosa, H.M., Carneiro, J.E.S. and Vieira, C. (1988). Ethyl methanesulfonate-induced seedcoat color mutants in *Phaseolus vulgaris* L. *Rev. Brasil. Genet. 11*: 123-128.

Carneiro, J.E.S., Barbosa, H.M., Vieira, C. and Cardoso, A.A. (1987). Alterações nos caracteres de plantas $M_1$ de *Phaseolus vulgaris* derivadas de sementes tratadas com etil-metanossulfonato. *Rev. Ceres* (Viçosa) *34*: 313-320.

Gaul, H. (1977). Mutagen effects in the first generation after seed treatment: Sterility. In: *Manual on Mutation Breeding*, 2nd edn., IAEA, Vienna, pp. 96-98.

Genter, C.F. and Brown, H.M. (1941). X-ray studies on the field bean. *J. Hered. 32*: 39-44.

Gottschalk, W. and Wolff, G. (1983). *Induced Mutations in Plant Breeding*. Monographs on Theoretical and Applied Genetics 7. Berlin, Springer-Verlag, 238 pp.

Guerra Chomon, J.C., Moh, C.C. and Alan, J.J. (1975). Inducción de mutaciones de color de semilla con metanosulfonato de etilo en algunas variedades de frijol común. *Turrialba 25*: 148-151.

Hänsel, H. (1968). An estimate of the rate of chlorophyll mutations in barley taking account of multiply mutated $M_1$-nuclei. In: *Mutations in Plant Breeding* II, IAEA, Vienna, pp. 139-150.

Hussein, H.A.S. and Disouki, I.A.M. (1976). Mutation breeding experiments in *Phaseolus vulgaris* (L.). I. EMS and gamma-ray-induced seed coat colour mutants. *Z. Pflanzenzüchtg. 76*: 190-199.

Kooiman, H.N. (1931). Monograph on the genetics of *Phaseolus*. *Bibliographia Genetica 8*: 295-413.

Lamprecht, H. (1935). Zur Genetik von *Phaseolus vulgaris*. XII. Uber die Vererbung der Blüten- und Stammfarbe. *Hereditas 21*: 129-166.

Moh, C.C. (1968). Bean mutant induced by ionizing radiation. III. Wrinkled leaf. *Turrialba 18*: 181-182.

Moh, C.C. (1969). Seed-coat color changes induced by ethyl methanesulfonate in the common bean (*Phaseolus vulgaris* L.). *Mutat. Res. 7*: 467-471.

Moh, C.C. (1971). Mutation breeding in seed-coat colors of beans (*Phaseolus vulgaris* L.). *Euphytica 20*: 119-125.

Moh, C.C. and Alan, J.J. (1964). Bean mutant induced by ionizing radiation. I. Dwarf mutant. *Turrialba 14*: 82-84.

Moh, C.C. and Alan, J.J. (1970). Bean mutant induced by ionizing radiation. V. Curly leaf. *Turrialba 20*: 120-121.

Moh, C.C. and Alan, J.J. (1971a). Correlation between seed-coat color and the seedling characters in *Phaseolus vulgaris* L. *Turrialba 21*: 173-175.

Moh, C.C. and Alan, J.J. (1971b). Bean mutant induced by ionizing radiation. VI. Unifoliate mutant. *Turrialba 21*: 231-232.

Moh, C.C. and Alan, J.J. (1971c). Bean mutant induced by ionizing radiation. VII. Compact mutant. *Turrialba 21*: 478-480.

Nilan, R.A. (1972). Mutagenic specificity in flowering plants: facts and prospects. In: *Induced Mutations and Plant Improvement*, IAEA, Vienna, pp. 141-150.

Nilan, R.A. and Konzak, C.F. (1961). Increasing the efficiency of mutation induction. In: *Mutations and Plant Breeding*, NAS/NRC Publ. 891: 437-458.

Nilan, R.A. and Vig, B.K. (1976). Plant test systems for detection of chemical mutagens. In: *Chemical Mutagens: Principles and Methods for Their Detection.* (Hollaender, A., Ed.), Vol. 4 Plenum Press, New York, pp. 143-170.

Prakken, R. (1934). Inheritance of colours and pod characters in *Phaseolus vulgaris* L. *Genetica 16*: 177-294.

Prakken, R. (1940). Inheritance of colours in *Phaseolus vulgaris* L. I. *Genetica 22*: 331-408.

Prakken, R. (1972). Inheritance of colours in *Phaseolus vulgaris* L. III. On genes for red seedcoat color and a general synthesis. *Meded. Landbouwhogesch. 72*: 1-82.

Swarup, V. and Gill, H.S. (1968). X-ray induced mutations in French bean. *Indian J. Genet. Plant Breed. 28*: 44-58.

Tara Mohan, S. (1979). EMS-induced dwarf mutant of dry beans (*Phaseolus vulgaris* L.). *Turrialba 29*: 317-318.

Vieira, C. (1967). *O Feijoeiro Comum: Cultura, Doenças e Melhoramento.* Viçosa, Imprensa Universitária, 220 pp.

**(Received April 27, 1988)**