

# Number of surviving initial cells and lack of haplontic selection in gamma ray-induced chimerism in the common bean, *Phaseolus vulgaris* L.

Hélio M. Barbosa<sup>1</sup> and Cláudio Coelho de Paula<sup>2</sup>

## ABSTRACT

Seeds of *Phaseolus vulgaris* L., cv. Milionário 1732, were obtained by planting seeds harvested from a single plant. They were treated with 20 krad from a <sup>60</sup>Co source and planted in the field. Seeds were harvested from each sector (branch or main stem) and grown as single sector M<sub>2</sub> progeny from each of 500 M<sub>1</sub> plants. The M<sub>3</sub> generation was grown from 11 segregating M<sub>2</sub> progenies. Segregation ratios for chlorophyll and seedcoat color mutants were scored in M<sub>2</sub> and M<sub>3</sub>. The objectives were a) to obtain additional information on the number of surviving initial cells from which a plant or sector is derived, and b) to determine whether or not the gametes carrying induced mutations are subject to haplontic selection. M<sub>1</sub> plants of Milionário 1732 grown from seeds treated with 20 krad of gamma rays originated from about five to nine cells. Each M<sub>1</sub> sector was derived from three cells. There was no evidence of haplontic selection.

## INTRODUCTION

In the common bean (*Phaseolus vulgaris* L.), as in most seed-propagated species, mutagenic treatments are usually applied to seeds. In these species, the shoot apical meristem contains a large number of cells, usually referred to as initial cells (Gaul, 1961; Muller, 1963; Beard, 1970), which originate the plant stem, leaves and reproductive organs. Besides inducing viable mutations, seed treatment with mutagens can cause cell injury, including death. Because of the multicellular nature of the shoot apex, an M<sub>1</sub> plant carrying a mutation will most probably be chimeric, i.e., have mutated and non-mutated sectors. For this reason, a deficit of mutants is usually found in segregating M<sub>2</sub>

progenies. Other possible reasons are diplontic selection (Gaul, 1961), i.e., selection against mutated somatic cells, and haplontic selection (Dellaert, 1980), which reduces the viability of gametes carrying the mutant allele.

The number of initial cells that survive the mutagenic treatment is the primary factor affecting the frequency of mutants recovered in M<sub>2</sub> progenies derived from chimeric plants. This number is usually estimated from data on M<sub>2</sub> segregation ratios of monogenic recessive chlorophyll mutations (Saccardo, 1983). Determinations of the number of initial cells have been made for several species (references in Beard, 1970 and Kawai, 1983). The present investigation was carried out a) to obtain additional information on the number of initial cells that survives the irradiation of dormant bean seeds, and b) to determine whether or not the gametes carrying the induced mutations were subject to haplontic selection.

<sup>1</sup> Departamento de Biologia Geral, Universidade Federal de Viçosa (UFV), 36571-000 Viçosa, MG, Brasil. Send correspondence to H.M.B.

<sup>2</sup> Departamento de Biologia Vegetal, UFV, 36571-000 Viçosa, MG, Brasil.

## MATERIAL AND METHODS

Seeds derived from a single plant of variety Milionário 1732 were planted for an additional generation to provide enough seeds for treatment. One thousand seeds (ca. 11.5% moisture) were irradiated with 20 krad from a  $^{60}\text{Co}$  source (dose rate of 3.07 krad/min) at CENA, Piracicaba, SP. Choice of radiation dose was based on previous results obtained with the same variety (Sena *et al.*, 1991). These seeds were planted 12 h later at Viçosa, MG, in 25 eight-meter rows, spaced 0.60 m apart, with 0.20 m between seeds in the row.

Nine hundred and fifty  $M_1$  plants were harvested. Of these, 500 plants that apparently had a larger number of branches and pods were selected to grow the  $M_2$  generation. From each of them, seeds were harvested from each sector (branch or main stem) and planted in separate sector progeny rows. Spacing was the same as before. Chlorophyll mutants were scored soon after emergence. At flowering time, the number of surviving plants was registered.

Each  $M_2$  progeny row was harvested separately and a sample of five pods was taken from each plant to score for possible seedcoat color mutants. Segregation ratios were determined by dividing the number of mutants by the number of  $M_2$  plants in the progeny of a single  $M_1$  plant or sector. Data on segregation ratios were taken for chlorophyll and seedcoat color mutants. The number of surviving initial cells was calculated by dividing the segregation ratio expected for a recessive mutation (25%) by the segregation ratio found (Kawai, 1983).

Ten  $M_2$  progenies that segregated for chlorophyll mutants and three that segregated for seedcoat color mutants were selected to grow the  $M_3$  generation. Preference was given to *albina* segregating progenies to facilitate mutant classification. From each  $M_2$  plant having a normal phenotype (either homozygous or heterozygous for the mutation) a sample of ten  $M_3$  seeds was taken and planted to grow a single  $M_3$  progeny row. Mutants were scored as in the previous generation. In view of the low number of seeds used to grow each  $M_3$  progeny, data from progenies segregating for the same mutant phenotype and derived from the same  $M_2$  (or  $M_1$ ) plant were combined and the chi-square test applied to detect possible deviations from the expected 3:1 ratio of normal to mutant. Evidence in favor of or against haplontic selection was obtained by determining whether or not there was a deficiency of mutants in segregating  $M_3$  progenies.

## RESULTS AND DISCUSSION

### Segregation ratios and number of initial cells

Thirty-seven  $M_2$  progenies (7.4%) segregated for chlorophyll mutations (*xantha*, *albina* or *viridis*) (Table I). Progenies of plants 124 and 209 each segregated for two different mutants. Segregation ratios varied from 0.73 to 13.41%, much less than the 25% ratio expected for a recessive single locus mutation. The chimeric nature of the  $M_1$  plant seems to be the major cause of mutant deficiency in segregating progenies (Gottschalk and Wolff, 1983).

The number of initial cells that survived the treatment varied from approximately two to 34 (Table I). Of the  $M_1$  plants that segregated in  $M_2$ , 38.5% descended from more than 10 initial cells, and 61.5% derived from two to 10 cells. The number of surviving initial cells ranged from about five, when estimation was based on pooled data of progenies segregating for *viridis*, to nine, when based on *xantha*-segregating progenies. After treating seeds with ethylmethanesulfonate, Motto *et al.* (1975) concluded that the bean plant descended from three to eighth initial cells. Using gamma rays, Barbosa and Sena (1992) reported that the bean plant derived from about two to nine cells. According to D'Amato (1965), deficits of recessives and thus lower segregation ratios are more frequently found with chemical mutagens than with radiation. As a result, the average number of cells tends to be higher in chemically treated material. Such estimations, however, may vary considerably depending on the mutagens and dose applied.

Similar studies based on segregation ratios per  $M_1$  plant sector (lateral branch or main stem) face the problem of a low number of available seeds (or plants), as pointed out by Barbosa and Sena (1992). Segregation ratios per sector (Table II) varied from 8.04% for total of progenies segregating for *albina*, to 9.21% for progenies segregating for *viridis*. These values indicate that each sector descended from about three surviving cells. Motto *et al.* (1975) reported that one to three cells were involved in the origin of a primary branch.

Only 3 out of 500  $M_1$  plants (0.6%) segregated for seedcoat color mutants (Table III). Segregation ratios based on pooled data were 2.91% when estimation was based on  $M_1$  plant progenies, and 6.15% when based on  $M_1$  sector progenies. These values indicate that each plant was derived from about eight to nine cells, and each sector descended from four cells, in good agreement with the estimates based on chlorophyll mutants (Tables I and II).

**Table I** - Segregation ratios and number of surviving initial cells, estimated from data on M<sub>1</sub> plant progenies segregating for chlorophyll mutations.

Identification No. of M <sub>1</sub> plant	No. of M <sub>2</sub> plants		No. of initial cells
	Total	Mutant	
<i>Xantha</i>			
12	91	2	11.4
36	91	6	3.8
66	106	4	6.6
124	57	1	14.3
166	115	3	9.6
209	92	3	7.7
210	88	1	21.9
220	83	1	20.8
239	76	4	4.8
306	54	2	6.8
417	118	1	29.4
425	137	1	34.2
443	54	4	3.4
Total	1162	33	8.8 <sup>1</sup>
<i>Albina</i>			
292	58	5	2.9
335	105	2	13.2
349	50	4	3.1
362	75	3	6.3
380	87	2	10.9
384	66	1	16.4
426	73	1	18.2
486	45	3	3.7
Total	559	21	6.6 <sup>1</sup>
<i>Viridis</i>			
69	67	2	8.4
124	57	3	4.8
132	53	3	4.4
147	82	11	1.9
157	61	4	3.8
165	51	2	6.4
207	53	1	13.2
209	92	8	2.9
214	66	1	16.4
218	48	1	12.0
286	46	1	11.5
346	48	1	12.0
383	74	5	3.7
394	61	2	7.6
399	37	1	9.3
418	80	2	10.0
439	57	2	7.1
478	77	3	6.4
Total	1110	53	5.2 <sup>1</sup>
Grand total	2831	107	6.6 <sup>1</sup>

**Table II** - Segregation ratios and number of surviving initial cells estimated from data on M<sub>1</sub> sector progenies segregating for chlorophyll mutations.

Identification No. of M <sub>1</sub> plant	No. of M <sub>2</sub> plants per sector		No. of initial cells
	Total	Mutant	
<i>Xantha</i>			
12-3	31	2	3.9
36-2	29	6	1.2
66-2	30	4	1.9
124-3	8	1	2.0
166-4	38	3	3.2
209-1	34	3	2.8
210-3	18	1	4.5
220-3	4	1	1.0
239-3	57	4	3.6
306-2	43	2	5.4
417-2	32	1	8.0
425-2	32	1	8.0
443-2	21	4	1.3
Total	377	33	2.9 <sup>1</sup>
<i>Albina</i>			
335-1	27	2	3.4
349-2	21	4	1.3
362-3	55	3	4.6
380-3	10	2	1.3
384-1	13	1	3.3
426-3	50	1	12.5
486-3	23	3	1.9
Total	199	16	3.1
<i>Viridis</i>			
69-3	29	2	3.6
124-4	22	3	1.8
132-3	35	3	2.9
165-4	23	2	2.9
207-1	16	1	4.0
209-1	34	8	1.1
214-2	18	1	4.5
218-2	6	1	1.5
286-1	31	1	7.7
346-3	28	1	7.0
383-4	45	5	2.3
394-3	21	2	2.6
399-1	4	1	1.0
418-2	24	2	3.0
439-4	44	2	5.5
Total	380	35	2.7 <sup>1</sup>
Grand total	956	84	2.8 <sup>1</sup>

<sup>1</sup>Calculation based on number of M<sub>2</sub> plants presented at left in the same row.<sup>1</sup>Calculation based on number of M<sub>2</sub> plants presented at left in the same row.

**Table III** - Segregation ratios and number of surviving initial cells based on M<sub>1</sub> plant progenies and M<sub>1</sub> sector progenies segregating for seedcoat color mutations.

Identification No. of		No. of M <sub>2</sub> plants per progeny of				No. of initial cells	
		M <sub>1</sub> plant		M <sub>1</sub> sector			
M <sub>1</sub> plant	M <sub>1</sub> sector	Total	Mutant	Total	Mutant	M <sub>1</sub> plant	M <sub>1</sub> sector
241	241-4	45	1	19	1	11.3	4.8
385	- <sup>1</sup>	104	2	-	-	13.0	-
439	439-4	57	3	46	3	4.8	3.8
Total		206	6	65	4	8.6 <sup>2</sup>	4.1 <sup>2</sup>

<sup>1</sup>Sector progeny not considered due to segregation ratio higher than 25%.<sup>2</sup>Calculation based on number of M<sub>2</sub> plants at left in the same row.

## Haplontic selection

Of 10 M<sub>2</sub> progenies that segregated for chlorophyll mutations, eight segregated in M<sub>3</sub>. Since the segregating M<sub>3</sub> progenies were derived from heterozygous non-chimeric M<sub>2</sub> plants, deficiency of mutants, if observed, may be taken as evidence in favor of haplontic selection. As can be seen in Table IV, such was not the case, however. Segregation of normal to chlorophyll mutant had a good fit to the 3:1 ratio expected for a monogenic recessive mutation, indicative of no selection against mutant gametes. The same conclusion is arrived at by examining data on segregation of normal (black) to mutant (brown) seedcoat color (Table IV). These results give support to the estimations of the number of initial cells presented previously. If haplontic selection was present, its effect would be to overestimate the number of cells. Haplontic selection has been detected in radiation-induced mutants of barley and *Arabidopsis* (Dellaert, 1980, and references therein). The absence of haplontic selection in the present study may be attributed to the low radiation dose applied, which resulted in 95% survival of M<sub>1</sub> plants, indicative of a low level of genetic damage.

## ACKNOWLEDGMENTS

The authors are indebted to Dr. A. Tulmann Neto (CENA, Piracicaba, SP), for providing facilities for irradiation of seeds.

Research supported by CAPES and CNPq.

## RESUMO

Sementes de feijão (*Phaseolus vulgaris* L.), cv. Milionário 1732, foram obtidas da multiplicação de sementes oriundas de uma única planta. Elas foram tratadas com 20 krad de radiação gama e plantadas no campo. De cada uma

**Table IV** - Segregation of normal to mutant in M<sub>3</sub> progenies.

Identification No. of M <sub>1</sub> plant	No. of M <sub>3</sub> plants		$\chi^2$ (Exp. 3:1) <sup>2</sup>	Probability
	Normal	Mutant <sup>1</sup>		
239	40	22 (x)	3.634	0.10-0.05
241	6	3 (s)	-	-
292	46	15 (a)	0.005	0.95-0.90
335	20	9 (a)	0.287	0.70-0.50
349	15	3 (a)	-	-
362	9	1 (a)	-	-
380	29	10 (a)	0.008	0.95-0.90
385	16	4 (s)	0.067	0.80-0.70
426	35	11 (a)	0.029	0.90-0.80
439	6	4 (s)	-	-
486	44	17 (a)	0.268	0.70-0.50

<sup>1</sup>a = Albina; s = seedcoat color mutant; x = xantha.<sup>2</sup>The chi-square test was not applied when the expected number of mutants was less than 5. Yates' correction factor was used when that number was between 5 and 10.

de 500 plantas M<sub>1</sub> foram colhidas sementes de cada setor (ramo ou haste principal) e plantadas em fileiras individuais de plantas M<sub>2</sub>. A geração M<sub>3</sub> foi obtida de 11 progênies M<sub>2</sub> segregantes. As segregações de mutantes clorofilianos e de mutantes da cor do tegumento da semente foram analisadas nas gerações M<sub>2</sub> e M<sub>3</sub>. Os objetivos foram: a) obter informação adicional sobre o número de células iniciais sobreviventes que originam uma planta ou um setor (ramo ou haste principal) e b) determinar se gametas portadores de mutações induzidas estão sujeitos à seleção haplôntica. Os resultados indicaram que a) uma planta de Milionário 1732 oriunda de semente tratada com 20 krad originou-se de cinco a nove células; b) cada setor originou-se de três células; e c) não houve evidência de seleção haplôntica.

## REFERENCES

- Barbosa, H.M. and Sena, J.S.P. (1992). Chimerism in bean (*Phaseolus vulgaris* L.) plants grown from irradiated seeds. *Rev. Bras. Genet.* 15: 419-427.

- Beard, B.H.** (1970). Estimating the number of meristem initials after seed irradiation: a method applied to flax stems. *Radiat. Bot.* 10: 47-57.
- D'Amato, F.** (1965). Chimera formation in mutagen-treated seeds and diplontic selection. *Radiat. Bot.* (Suppl.) 5: 303-316.
- Dellaert, L.M.W.** (1980). Segregation frequencies of radiation-induced viable mutants in *Arabidopsis thaliana* (L.). Heynh. *Theor. Appl. Genet.* 57: 137-143.
- Gaul, H.** (1961). Studies on diplontic selection after X-irradiation of barley seeds. In: *Effects of Ionizing Radiation on Seeds*. IAEA, Vienna, pp. 117-138.
- Gottschalk, W. and Wolff, G.** (1983). *Induced Mutations in Plant Breeding. Monographs on Theoretical and Applied Genetics.* 7. Berlin, Springer-Verlag, pp. 238.
- Kawai, T.** (1983). M<sub>1</sub> chimerism following mutagen treatment of rice and some other cereals. IAEA-TECDOC-289, Vienna, pp. 7-11.
- Motto, M., Soressi, G.P. and Salamini, F.** (1975). Mutation frequencies and chimeric formation in *Phaseolus vulgaris* after EMS treatment of dormant seeds. *Radiat. Bot.* 15: 291-299.
- Muller, A.J.** (1963). The chimerical structure of M<sub>1</sub> plants and its bearing on the determination of mutation frequencies in *Arabidopsis*. In: *Induction of Mutations and the Mutation Process* (Velemínský, J. and Gichner, T., eds.). Proceedings of a Symposium, Prague, pp. 46-52.
- Saccardo, F.** (1983). Chimera formation in M<sub>1</sub> pea plants raised from mutagen-treated seeds. IAEA-TECDOC-289, Vienna, pp. 23-24.
- Sena, J.S.P., Barbosa, H.M. and Vieira, C.** (1991). Induced mutations in the common bean, *Phaseolus vulgaris* L., affecting flower color and seed characteristics. *Rev. Bras. Genet.* 14: 1033-1039.

(Received June 9, 1995)