

## CHIMERISM IN BEAN (*Phaseolus vulgaris* L.) PLANTS GROWN FROM IRRADIATED SEEDS

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### ABSTRACT

Seeds of the common bean (*Phaseolus vulgaris* L.) with ca. 13% moisture were treated with 0 (control), 6, 12, 18, 24, and 30 krad of gamma rays and planted in the field. Single plant progenies were grown in the M<sub>2</sub> generation, except for the 18-krad treatment. In this case, individual branch progenies were grown. The scoring of chlorophyll mutations in the M<sub>2</sub> generation led to the conclusion that the M<sub>1</sub> bean plant is derived from about two to nine initial cells of the embryo. More than one cell is probably involved in a primary branch formation. Additional data are needed to decide whether or not the mutated sectors are randomly distributed on the M<sub>1</sub> plant.

### INTRODUCTION

In sexually propagated crops seeds are the preferred material to be treated in mutation breeding programs (Nilan *et al.*, 1977; Brock, 1980; Gottschalk and Wolff, 1983). When seeds are treated with mutagens a mutation may be induced in only one of the many cells of the embryo. Plants grown from such seeds will thus have mutated and non mutated sectors, i.e., will be chimeric. Chimerism creates difficulties for the recovery of mutants since the mutated sector may not be represented in the seed sample drawn to grow the M<sub>2</sub> generation. As a consequence, a deficit of recessives will be found in the M<sub>2</sub> generation. Knowledge of the chimeric structure of the M<sub>1</sub> plant may contribute to a better understanding of ontogenetic processes (D'Amato, 1965) and to a more efficient

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sampling procedure (Lindgren *et al.*, 1970). Much more is known about chimera formation in cereal than in leguminous species (IAEA, 1983).

The present study was initiated to obtain information on the chimeric structure of bean (*Phaseolus vulgaris* L.) plants derived from gamma ray-treated seeds. In this species such studies are particularly scarce.

## MATERIALS AND METHODS

Gamma ray doses of 0 (control), 6, 12, 18, 24, and 30 krad were applied to 300-seed lots (ca. 13% moisture) of *P. vulgaris* L., cv. Milionário 1732 (BAT 65). The dose rate was 3.5 krad/min. Planting was made in the field at Viçosa, MG, in a randomized complete block design with six replications. Each plot consisted of two rows 5 m long, 60 cm apart, with seed hills spaced 20 cm.

All M<sub>1</sub> plants were harvested separately. Those derived from seeds treated with 6, 12, 24, and 30 krad were threshed individually and from each a maximum of 70 seeds was used to grow the M<sub>2</sub> generation. From each M<sub>1</sub> plant derived from seeds treated with 18 krad individual branches were threshed and their position on the plant recorded. A maximum of 20 seeds from each M<sub>1</sub> branch was taken to grow the M<sub>2</sub> generation. From the non irradiated control 70 seeds were planted in each of 50 rows, uniformly distributed among the M<sub>2</sub> populations.

Chlorophyll mutants were recorded as soon as germination started. Two methods were used to study the chimeric structure of the M<sub>1</sub> plants. The first, proposed by Weiling and Gottschalk, cited by Saccardo (1983), is based on the segregation ratio of a single recessive chlorophyll mutation in the M<sub>2</sub> generation. The expected segregation ratio in M<sub>2</sub> for a recessive mutation in a non-chimeric M<sub>1</sub> plant is 25%. The number of functional initial cells of the apical meristem which give rise to the adult M<sub>1</sub> plant was calculated by dividing the expected (25%) by the observed segregation ratio for a recessive mutation in M<sub>2</sub> (Kawai, 1983). The observed segregation ratio is the frequency of recessive chlorophyll mutants actually found in M<sub>2</sub>. The second method is based on the topographical analysis devised by D'Amato (1965) and localizes the mutation in the M<sub>1</sub> plant. In the present study mutations induced by the 18-krad treatment could be localized in the respective M<sub>1</sub> branch by diagrammatically describing the M<sub>1</sub> plant and growing single branch progenies.

## RESULTS AND DISCUSSION

The progenies that segregated for chlorophyll mutations are presented in Table I. Only four M<sub>2</sub> progenies segregated close to the expected ratio of 3 normal:mutant: 1011-1012 ( $\chi^2 = 0.035$ ;  $0.80 < P < 0.90$ ), 1261-1263 ( $\chi^2 = 0.333$ ;  $0.50 < P < 0.70$ ), 607

( $\chi^2 = 0.12$ ;  $0.70 < P < 0.80$ ), and 627 ( $\chi^2 = 0.178$ ;  $0.50 < P < 0.70$ ). The  $M_1$  plants which gave rise to these progenies were certainly non-chimeric, that is, they were derived from a single cell heterozygous for the mutation, the other cells of the embryo having been killed by the irradiation. Nine  $M_2$  progenies (17%) segregated for two chlorophyll mutants (*albina* and *xantha* or *viridis* and *xantha*). In *Vicia faba*, Hermelin *et al.* (1983) reported 21% of the  $M_2$  progenies segregating for more than one mutant phenotype (chlorophyll and morphological). In the majority of the progenies, however, there was a deficit of mutants, as expected.

Table I - Segregation for chlorophyll mutations in  $M_2$  progenies.

Radiation dose (krad)	Identification of $M_2$ progeny	No. of plants in $M_2$ progeny		Mutant phenotype
		Normal	Mutant	
0 (control)	several	all	-	-
6	55	52	2	1 <i>viridis</i> , 1 <i>xantha</i>
	67	53	1	<i>albina</i>
	92	50	1	<i>viridis</i>
	136	55	1	<i>viridis</i>
	144	49	1	<i>viridis</i>
	151	57	3	<i>viridis</i>
	160	40	1	<i>viridis</i>
	203	45	2	<i>xantha</i>
	215	43	1	<i>viridis</i>
	246	47	1	<i>albina</i>
12	286	40	3	1 <i>albina</i> , 2 <i>xantha</i>
	297	53	3	<i>viridis</i>
	313	38	2	1 <i>albina</i> , 1 <i>xantha</i>
	343	55	1	<i>xantha</i>
	348	16	3	<i>viridis</i>
	352	37	3	<i>xantha</i>
	354	56	1	<i>xantha</i>
	356	43	1	<i>xantha</i>
362	48	4	<i>viridis</i>	

Continued

Table I - Continued

Radiation dose (krad)	Identification of M <sub>2</sub> progeny	No. of plants in M <sub>2</sub> progeny		Mutant phenotype
		Normal	Mutant	
	376	35	1	<i>xantha</i>
	385	35	5	1 <i>albina</i> , 4 <i>xantha</i>
	399	53	1	<i>viridis</i>
	422	42	2	<i>albina</i>
	432	54	2	<i>viridis</i>
	433	39	4	1 <i>viridis</i> , 3 <i>xantha</i>
	438	39	3	1 <i>albina</i> , 2 <i>xantha</i>
	453	35	1	<i>xantha</i>
18	838-841	52	1	<i>viridis</i>
	882-884	32	1	<i>viridis</i>
	964-966	50	1	<i>albina</i>
	973-976	59	1	<i>albina</i>
	1011-1012	29	9	<i>albina</i>
	1018-1021	34	4	3 <i>albina</i> , 1 <i>xantha</i>
	1041-1042	31	1	<i>xantha</i>
	1081-1085	39	4	<i>viridis</i>
	1150-1153	66	1	<i>xantha</i>
	1163-1165	37	3	1 <i>albina</i> , 2 <i>xantha</i>
	1167-1171	57	1	<i>xantha</i>
	1172-1175	50	2	<i>viridis</i>
	1234-1236	37	2	<i>viridis</i>
	1250-1254	80	5	<i>viridis</i>
	1261-1263	20	5	<i>viridis</i>
	1385-1387	37	4	<i>xantha</i>
	1399-1402	54	1	<i>xantha</i>
24	543	21	1	<i>viridis</i>
	551	20	1	<i>xantha</i>
	556	26	2	<i>albina</i>
	570	33	1	<i>albina</i>

Continued

Table I - Continued

Radiation dose (krad)	Identification of M <sub>2</sub> progeny	No. of plants in M <sub>2</sub> progeny		Mutant phenotype
		Normal	Mutant	
	583	52	1	<i>xantha</i>
	594	35	5	1 <i>viridis</i> , 4 <i>xantha</i>
	597	59	8	<i>viridis</i>
	607	53	16	<i>xantha</i>
	627	34	13	<i>albina</i>
30	several	all	-	-

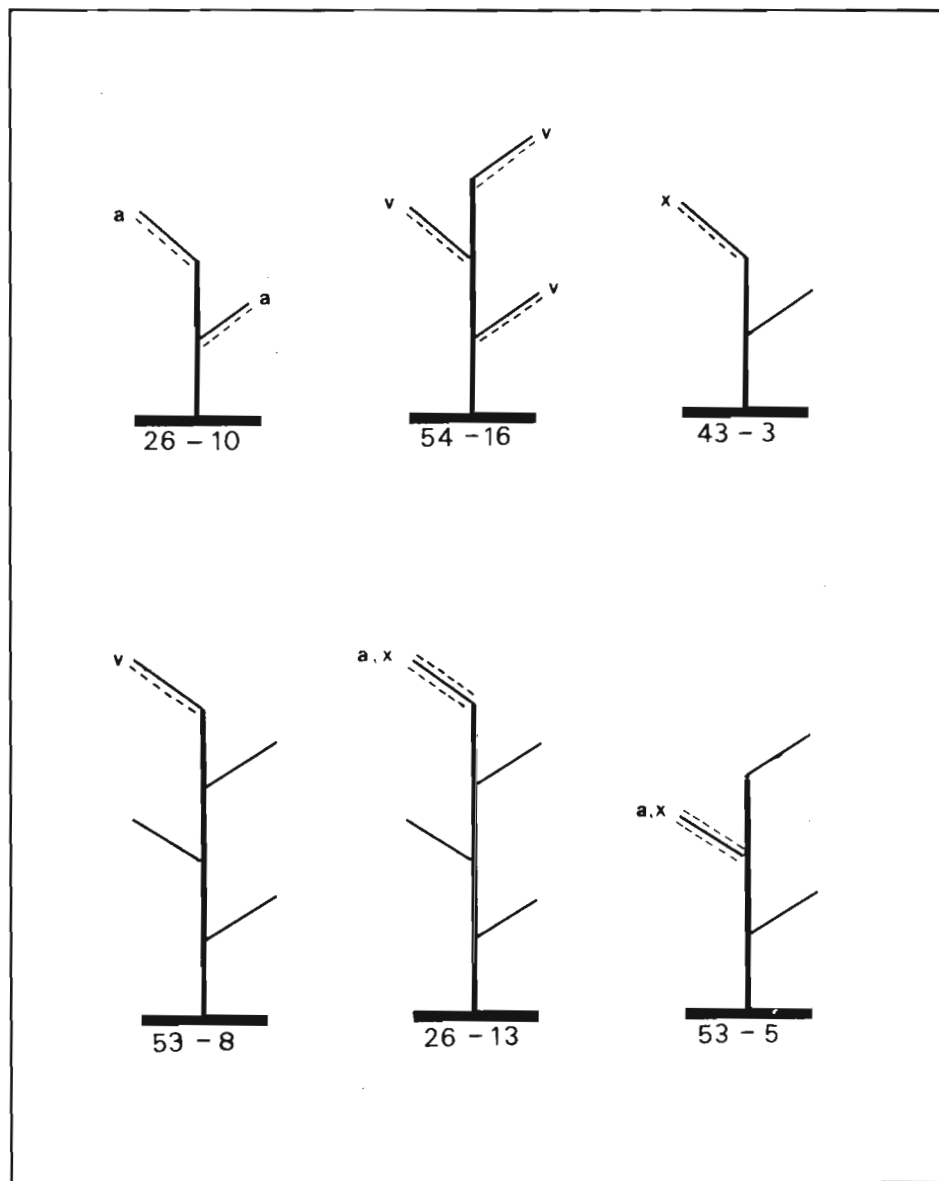
Assuming no selection against cells carrying the mutation and excluding the progenies segregating for two chlorophyll mutations, one can determine the number of initial cells making up the shoot apex (Table II). The calculation is based on the segregation ratio for a monogenic recessive mutation in M<sub>2</sub>. Since the expected segregation ratio for a recessive mutation in the progeny of a non-chimeric M<sub>1</sub> plant is 25%, if two cells give rise to the plant reproductive organs, and one of them carries the mutation, the expected segregation ratio will be 12.5%. By similar reasoning one can determine the number of initial cells that gave rise to the plant as the ratio of the expected to the observed segregation ratio. As seen in Table II, the number of initial cells varied from about two to nine, decreasing with increasing radiation dose. Such a reduction in cell number is caused by the higher probability of cell mortality with increasing doses. Cell elimination by higher doses of radiation increases the segregation ratio due to the increased size of the mutated sector. After treating bean seeds with EMS, Motto *et al.* (1975) reported that three to eight cells gave rise to the entire plant, in agreement with the present results. However, they did not find an increased segregation ratio as the EMS concentration increased. Results of studies on chimeric structure seem to depend on the mutagen and dose employed (Kawai, 1983) and, possibly, on the variety used (D'Amato, 1965). A complicating factor which affects the calculation of the number of initial cells is the possibility of occurrence of diplontic and/or haplontic selection (Gaul, 1961), caused by a competitive disadvantage of cells carrying the mutation. Evidence favoring selection against mutated cells has been reported by some authors (Gaul, 1961; Dellaert, 1980) but not by others (Yamaguchi, 1962; Muller, 1963; Lindgren *et al.*, 1970; Hermelin *et al.*, 1983). In beans, Motto *et al.* (1975) found no evidence of diplontic selection. If selection

against mutated cells occurs, it will decrease the mutated sector size. As a consequence, the number of initial cells will be overestimated.

Table II - Segregation ratio for chlorophyll mutations in the M<sub>2</sub> generation and number of initial cells as a function of radiation dose.

Radiation dose (krads)	No. of M <sub>2</sub> plants in progenies segregating for a single mutant		Segregation ratio	No. of initial cells
	Total	Mutant		
0	2674	0	-	-
6	451	12	2.661	9.39
12	550	23	4.182	5.98
18	732	39	5.328	4.69
24	341	43	12.610	1.98
30	585	0	-	-

Topographical analysis was made using single branch progenies derived from the 18-krad treatment. Figure 1 illustrates the chimeric structure of some M<sub>1</sub> plants. M<sub>1</sub> plants were found carrying the chlorophyll mutation in all branches (plants no. 26-10 and 54-16). The segregation ratio in the M<sub>2</sub> progenies from such plants was close to 25%, indicating a single cell origin. M<sub>1</sub> plants having two different chlorophyll mutations (*albina* and *xantha*) in the same branch were also found (plants no. 26-13 and 53-5). Such cases suggest that either a single cell, from which the branch was derived, carried two mutations, or more than one cell, each carrying a different mutation, gave origin to the same branch. Although the second possibility seems more probable, the induction of several mutations in a single cell of an embryo occurs relatively frequently in mutation induction experiments (Gottschak and Wolff, 1983). Plants having a single mutation in only one branch were more frequently found, the other branches giving rise to normal plants (plants no. 43-3 and 53-8). If each primary branch originated from a single initial cell, plants grown from its seeds should have yielded a segregation ratio close to 25%. Since this was not found, there having been a deficit of mutants, more than a single cell must have contributed to each branch formation. Motto *et al.* (1975) reported that one to three cells are probably involved in primary branch origin. However, inferences regarding the number of initial cells giving rise to a branch should be made with caution in view of the small number of M<sub>2</sub> plants derived from a single branch of a bean plant.

Figure 1 - Diagrams of some chimeric  $M_1$  plants.

- non mutated branch;
- branch carrying a single chlorophyll mutation;
- ===== branch carrying two chlorophyll mutations.

a - *albina*; v - *viridis*; x - *xantha*

Seventeen plants (6.77% of the surviving  $M_1$  plants) grown from seeds treated with 18 krads segregated for chlorophyll mutations in  $M_2$ . Their chimeric patterns were as follows: a) twelve plants segregated for a single chlorophyll mutant in the  $M_2$  progeny of only one branch; b) three plants carried a single mutation in all branches; and c) two plants carried two different chlorophyll mutations in a single branch. The progeny of each  $M_1$  branch was examined to determine the frequency with which the mutation occurred in each branch, from the first (closest to the ground) to the last (farthest from the ground). About half of the mutations were carried in the second branch, mutations in the other branches occurring in about equal frequencies. Although the data suggest that seed harvest should be made from the second  $M_1$  branch, a definite conclusion regarding the best sampling procedure awaits additional data, due to the relatively low number of chimeric plants studied.

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### RESUMO

Sementes de feijão com cerca de 13% de umidade foram irradiadas com 0 (controle), 6, 12, 18, 24 e 30 krads de radiação gama e plantadas no campo. A geração  $M_2$  foi obtida plantando-se sementes de cada planta  $M_1$  separadamente, exceto para o tratamento com 18 krads. Neste caso, foram plantadas sementes de cada ramo separadamente. A ocorrência de mutantes clorofilianos na geração  $M_2$  permitiu determinar que duas a nove células iniciais do embrião estão envolvidas na formação da planta  $M_1$ . Cada ramificação primária origina-se de mais de uma célula. Dados adicionais são necessários para se determinar se os setores mutados na planta  $M_1$  ocorrem aleatoriamente.

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